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BIOLOGICAL BULLETIN

THE ODD CHROMOSOME IN CERASTIPSOCUS VENOSUS.[1]

ALICE M. BORING.

Many groups of insects belonging to the species *Cerastipsocus venosus* were found on one ash tree near the laboratory at Woods Hole last summer. As they belong to the Corrodentia, an order of insects in which the chromosome history has not yet been worked out, it seemed worth while to study the spermatogenesis. They are gregarious in habit and not strong flyers. In the middle of August there were still many nymphs, but they all soon developed wings and by September 1 had all flown from the ash tree. The material used was partly from nymphs and partly from adults. The males are slightly smaller than the females, and both nymphs and adults furnished the entire series of stages for the study of the spermatogenesis. The spermatogenesis falls into line with that of many insects in other orders. There is an odd chromosome that does not divide in the first spermatocyte division.

Methods.

The testes are small trilobed organs, resembling clover leaves, situated dorsally, one each side of the anterior end of the abdomen. The most of this study was made from acetocarmine preparations, but compared with material fixed in Gilson or Flemming and stained in iron hæmatoxylin or thionin. The small testis was put whole in a drop of Schneider's acetocarmine on a slide, left there a couple of minutes, covered with a coverglass, pressed flat, all extra acetocarmine was drawn off with

[1] I wish to express my thanks to Dr. Pearl for criticizing the manuscript of this and the following paper, to the Director of the Marine Biological Laboratory for the facilities offered me at Woods Hole, and to Mr. Nathan Banks and Mr. E. P. Van Duzee for identifying the species.

filter paper until the edges of the coverglass were dry, and then the edges were covered with vaseline to prevent evaporation. The value of such preparations has already been pointed out many times by N. M. Stevens. They can be used immediately and will often last a couple of weeks. All chromatic structures are distinctly fixed and stained, and some achromatic structures also. The size of the cells is much larger than in preserved material, due partly, as shown by Dr. Stevens,[1] to actual swelling in the acetic acid, and partly to the avoiding of the shrinkage in alcohol and xylol (Figs. 3 and 4). This method insures the study of whole cells and avoids any danger of missing parts of a cell cut off in other sections. If the material is properly pressed out under the coverglass, there will be only a single layer of cells. All the drawings but one in this paper were made from such preparations.

Observations.

In each lobe of the testis, the stages of spermatogenesis are arranged in a series from the distal end to the end joining the duct. The spermatogonial number is 17 (Fig. 1). Spermatogonial plates were not frequent but this number was counted clearly in four plates. The size of chromosomes varies somewhat but not sufficiently to study chromosome individuality. Not even the odd chromosome can be identified in the spermatogonia.

The odd chromosome differentiates itself first in the growth stages of the primary spermatocytes. In the early spermatocytes there is a long piece of the spireme lying close against the nuclear wall, that stains darker than the rest (Fig. 2). This next becomes more condensed and shorter, appearing as several beads. Some of the different forms are shown in Fig. 5. Finally it becomes a solid round intensely staining body in the midst of a nucleus with a very lightly staining spireme (Fig. 3). It sometimes retains, however, a slightly elongated shape in prophase (Fig. 6) and even on the spindle of the first spermatocyte division (Fig. 8). A plasmosome is also present during these growth stages.

In the stages of the first spermatocyte division, the odd chromosome is easily distinguishable from the others by its single condi-

[1] Stevens, N. M., 1908, "A Study of the Germ Cells in Certain Diptera," *Jour. Exp. Zoöl.*, V., p. 359.

tion. In prophase there are 8 bivalent chromosomes, appearing as dumbbells or tetrads while one odd chromosome remains round or slightly oblong with no signs of approaching division (Figs. 6 and 7). In metaphase as the dumbbells and tetrads become grouped at the equator of the spindle, the odd chromosome often still lags to one side of the equator (Figs. 8 and 9). In all these prophase and metaphase groups, the chromosomes are so large and distinct that there is no difficulty in being sure of the full number, 8 dumbbells or tetrads and one odd chromosome with no sign of division. The dumbbells are probably side views of the tetrads, as Fig. 8 shows nearly all tetrads. In the equatorial plates, the odd chromosome appears off to one side (Fig. 10). In anaphase, it sometimes precedes (Fig. 11), and sometimes follows (Fig. 12) the other chromosomes to one pole of the spindle. Fig. 13 shows the telophase of this division: the odd chromosome stands at one end of the spindle in one secondary spermatocyte, with no mate in the corresponding secondary spermatocyte. In Fig. 14, the chromosomes can be counted in the two reconstructing nuclei of the two cell products of one primary spermatocyte division: 8 dumbbells on one side, 8 dumbbells and one single odd chromosome on the other. Each dumbbell in the secondary spermatocyte represents half of a tetrad. Thus the plane of the second spermatocyte division was indicated already in the prophase of the first spermatocyte division, and still is in the prophase of the second, but there is no way to tell which is the reducing division. Among these telophase stages in one individual were a few abnormally large cells in which the mitotic mechanism had somewhere failed, and there was an unreduced number of chromosomes (Fig. 15). Here there are 16 dumbbells and the odd chromosome is recognizable from its round shape and its position to one side of the group. By the time the odd chromosome gets into the equatorial plate for the second spermatocyte division it is also a dumbbell in shape and cannot be distinguished from the others. Nothing but dumbbells appear on the secondary spermatocyte spindle, so the odd chromosome must divide in this division (Fig. 16). The equatorial plates of the secondary spermatocytes show the expected difference in the number of chromosomes, some 8

(Fig. 17), and some 9 (Fig. 18), but the odd chromosome is indistinguishable by either size or position. The anaphases show no chromosome lagging behind or behaving in any way differently from the others.

The behavior of the odd chromosome necessitates the conclusion that *Cerastipsocus venosus* has dimorphic spermatozoa. If half of the secondary spermatocytes have one more chromosome than the others, the spermatids and spermatozoa developing from them must also have one more chromosome. The chief interest in this study lies in the fact that it shows the existence of an odd chromosome in a species of the Corrodentia, an order in which it has not before been described, and therefore adds another order of insects to the list of those with sex chromosomes.

Summary.

1. The spermatogonial number of chromosomes is 17.

2. The odd chromosome is condensed in the growth stage, first as a long beadlike structure, later as one round body.

3. There are 9 chromosomes in the primary spermatocytes, 8 bivalent and one undivided odd chromosome.

4. The odd chromosome does not divide in the first spermatocyte division.

5. There are 9 chromosomes in half of the secondary spermatocytes and 8 in the other half.

6. All the chromosomes, including the odd chromosome, divide in the second spermatocyte division.

7. Therefore, this species of Corrodentia has dimorphic spermatozoa.

University of Maine, Orono,
November, 1912.

All figures were drawn with a camera lucida, 1/12 oil immersion and a 12 compensating ocular, no reduction. All drawings but Fig. 4 are from acetocarmine preparations.

Explanation of Plate I.

Fig. 1. Metaphase of spermatogonium, showing 17 chromosomes.

Figs. 2, 3. Spermatocyte growth stages, showing plasmosome (*P*) and two stages of condensation in the odd chromosome (*X*).

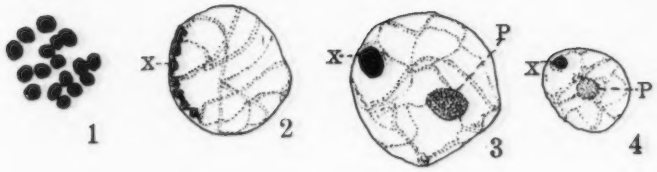
Fig. 4. Same stage as 3, but from permanent preparation fixed in Gilson.

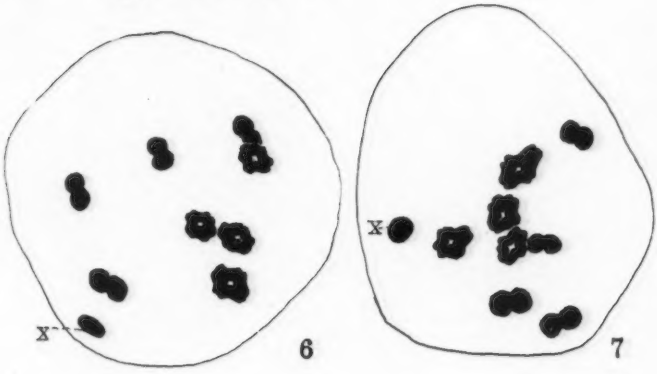
Fig. 5. Odd chromosome in different stages of growth.

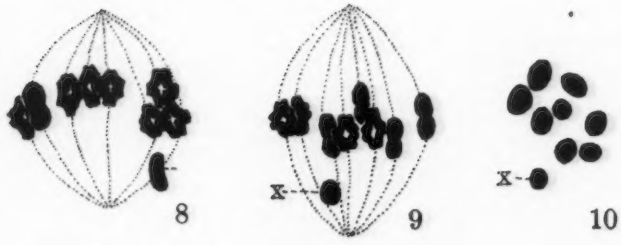
Figs. 6, 7. Prophases of primary spermatocytes, 8 bivalents and 1 single odd chromosome (*X*).

Figs. 8, 9. Metaphase spindles of primary spermatocytes, odd chromosome (*X*) toward one pole.

Fig. 10. Equatorial plate of primary spermatocyte, 9 chromosomes, odd chromosome (*X*) to one side of the group.







A. M. BORING.

Explanation of Plate II.

Figs. 11, 12. Anaphases of primary spermatocyte division, odd chromosome (X) undivided at one pole.

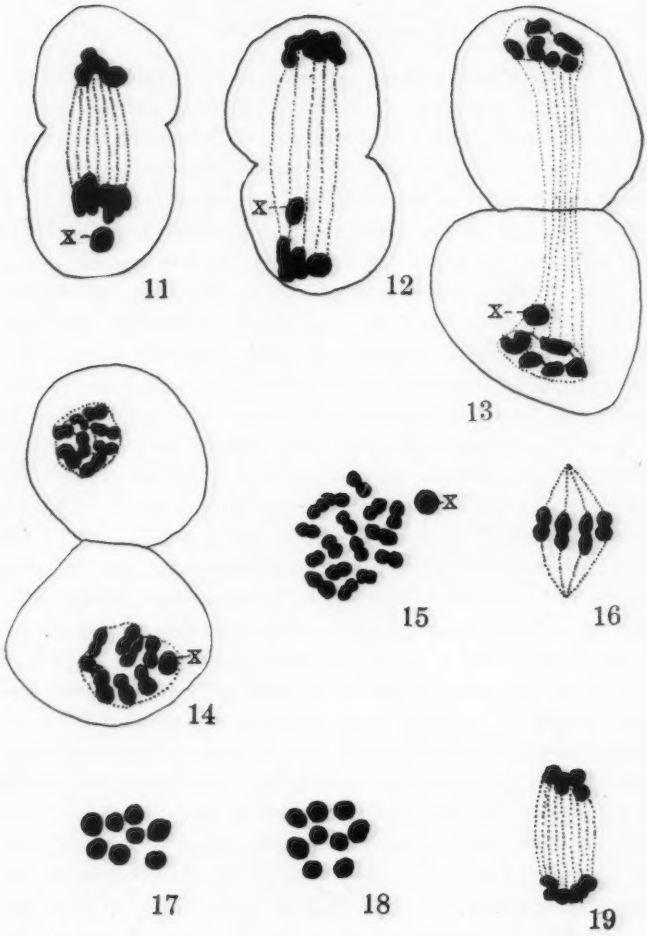
Figs. 13, 14. Two pairs of secondary spermatocytes, X present in only one of each pair.

Fig. 15. Abnormal secondary spermatocyte, 16 chromosomes and 1 odd chromosome (X).

Fig. 16. Metaphase of secondary spermatocyte, all chromosomes dividing.

Figs. 17, 18. Equatorial plates of secondary spermatocytes, one with 8, the other with 9 chromosomes.

Fig. 19. Anaphase of secondary spermatocyte, no lagging chromosome.



A. M. BORING.

THE CHROMOSOMES OF THE CERCOPIDÆ.

ALICE M. BORING.

The chromosomes of at least four species of this family have been already studied, one by Dr. N. M. Stevens,[1] and three by the writer.[2] These three species which I have already studied were collected at Cold Spring Harbor in 1907 and identified by Mr. E. P. Van Duzee as *Clastoptera obtusa*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. The species studied by Dr. Stevens was collected at South Harpswell in 1906 and identified at the time as *Aphrophora quadrangularis*, but the cytological differences from the Cold Spring Harbor material which was surely *Aphrophora quadrangularis* made Dr. Stevens later question the identification of the Harpswell material. The reduced number of chromosomes is different in all of these species, 12 in the Harpswell form, 15 in *Clastoptera obtusa*, 11 (12?) in *Aphrophora quadrangularis*, and 14 in *Aphrophora quadrinotata*. In other respects, the spermatogenesis in one species resembles that of the others, except for the Harpswell form which differs in several points: the odd chromosome of the early growth stages of the primary spermatocytes is long and rodlike; in later stages two M-chromosomes appear; and the odd chromosome in the equatorial plates of the primary spermatocytes is one of the medium-sized chromosomes. In the other species, the odd chromosome in the growth stages is never rodlike, but always rounded, and no M-chromosomes ever appear in the growth or division stages; the odd chromosome in the equatorial plates of the first spermatocyte division and in the sideview of the spindles appears to be one of the smaller chromosomes. In 1909, I collected material of the common European spittle insect, *Aphrophora spumaria*, at Eisenach, for chromosome study, and this summer at Woods Hole a chance to study one more species of this family was

[1] Stevens, N. M., 1906, "Studies in Spermatogenesis," Part II., Carnegie Inst., Washington.

[2] Boring, A. M., 1907, "A Study of the Spermatogenesis in Twenty-two Species of the Membracidæ," etc., *Jour. Exp. Zoöl.*, IV., p. 469.

afforded me by the abundance of *Philænus spumarius* on the goldenrod and wild sunflower. The results of the study of these last two species are given in this paper.

Material and Methods.

The *Philænus* material used in this study was found on the goldenrod and wild sunflower in the Fay woods at Woods Hole early in September. The adults were emerging rapidly from the nymph stage and the variation in marking was so striking that it seemed possible that the insects might belong to more than one species. However, specimens showing this variation were sent to Mr. E. P. Van Duzee, and he identified them all as *Philænus spumarius*. The same variations occurred among the insects on both goldenrod and wild sunflower. Nymphs from the two plants were kept separate in the laboratory until the adults appeared.

The *Aphrophora spumaria* material was collected from the grasses of a low meadow near Eisenach in September. All were adults at that time. This species exhibited as great somatic variations as *Philænus spumarius*, both in wing markings and the color of the abdomen.

The *Philænus* material used was partly from adults and partly from nymphs. The position and shape of the testes was exactly like that described for the material of the other species of Cercopidæ. In the just-emerged adults the testes show all stages of spermatogenesis, including fully formed spermatozoa, but in the females the ovaries are only slightly developed and difficult to find. There are only oögonia and very young growth stages of the primary oöcytes present. Evidently the eggs are not laid until the next spring.

For preserved material, the few posterior segments of the abdomen containing the testes were cut out and fixed in Gilson or in Flemming. But the most of the study of *Philænus* was made from acetocarmine preparations, and all the figures are drawn from such. This greatly expedited the study of the chromosomes in different individuals in connection with somatic variation. *Aphrophora spumaria* was studied entirely from preserved material.

Observations on Chromosome Individuality.

The chromosomes of *Philænus* are more like those of the three forms studied at Cold Spring Harbor than like the one worked on by Dr. Stevens at Harpswell. The abundance of the material and the large size of the chromosomes in the aceto-carmine preparations have made it possible to study the individuals in this form more accurately than previously in the other forms.

The equatorial plates of the primary spermatocytes always show 12 chromosomes (Figs. 1–7) and the secondary spermatocytes 11 and 12 (Figs. 8 and 9). The odd chromosome usually stands to one side of the primary spermatocyte plate, when it is in the same plane (Figs. 1–7, *X*), but it very often lies in an entirely different focus, at the end of the spindle (Figs. 10, 11, 12). It is always one of the smallest chromosomes. It is impossible to individualize each of the twelve chromosomes, but each plate shows one largest (*A*), two almost as large (*B*, *C*), several intermediate, and three or four smallest, one of which is the odd chromosome. The secondary spermatocyte plates show the same condition, one largest chromosome and two almost as large, but here in the plates with 11 chromosomes (Fig. 9), there is, of course, no odd chromosome and in the plates with 12 (Fig. 8), it has no particular position by which it can be identified.

The individuality of these chromosomes can be traced also in the prophase of the first spermatocyte division (Fig. 15). The odd chromosome is a single round body, while the others are already split for the first spermatocyte division. Among these dumbbell-shaped bodies the one largest and the two chromosomes almost as large are clearly recognizable (Fig. 15, *A*, *B*, *C*). In the side view of the metaphase figure the odd chromosome still appears round and undivided alongside of the other dividing chromosomes (Fig. 16). It is here about the size of half of one of the smallest bivalent chromosomes, so we could expect it to be undifferentiated by size from the smallest ones in the end view of the equatorial plates, as has been previously described.

In anaphase, the odd chromosome lags behind the others, but goes undivided to one pole. Its small size is again evident here (Fig. 17). The second spermatocyte division follows close after

the first. Fig. 18 shows the two chromosome groups from the ends of a spindle. Each chromosome is splitting, preparatory to the second division, as is also the odd chromosome. The two groups were not in the same focus, and it was clear that the odd chromosome belonged to the lower group. The size relations here are as before, one largest chromosome (*A*) in each group, and two almost as large (*B*, *C*). The odd chromosome (*X*) here appears slightly larger than the smallest, but they are rearranging themselves in shape and position for the second division, and therefore we cannot be sure that we are focusing on similar sides.

The equatorial plates in the spermatogonia and in the follicle cells are interesting to compare with those in the spermatocytes, for in both of the former, the unreduced number of chromosomes is present. The grouping in the spermatogonia is more compact than in the somatic cells (Figs. 19, 20), but in both there are two longest chromosomes (*AA*) and four almost as long (*BB*, *CC*), the three pairs represented by the one largest and two almost as large of the spermatocytes. In both also there is an odd number of chromosomes, 23, the condition invariably found in the spermatogenesis of forms with an odd chromosome. Which of the smaller chromosomes here is the odd one, there is no way to detect. In one somatic cell where the chromosomes are much longer and thinner than in any others (Fig. 22), the size relations are not so clear: there are two distinctly longest (*AA*), but apparently six next longest. However, the chromosomes are so long and twisted in this case that there is every possibility of having misrepresented their lengths in a flat drawing.

The equatorial plates of the oögonia and female somatic cells are similar to the corresponding male cells, except for the possession of one more chromosome, 24 instead of 23 (Figs. 23–25). This additional chromosome must be a small one, for there are only two longest (*AA*) chromosomes and four almost as long (*BB*, *CC*), as in the male cells. Presumably then this extra chromosome is the mate of the small odd chromosome of the male. Therefore, *Philænus spumarius* has dimorphic spermatozoa, and one more chromosome in all female cells than in the male cells, one usual expression of sex differentiation in insects.

Aphrophora spumaria has one chromosome distinctly larger than the others in the equatorial plates (Figs. 26–29). Sometimes there appear to be two intermediate-sized as in *Philænus*, but the cells and chromosomes are smaller and it is consequently not possible to be sure of small differences. The reduced number of chromosomes is 12, and one of these is an odd chromosome, which behaves in the typical way in division, like *Philænus* (Fig. 33, *X*).

The Odd Chromosome in the Growth Stages.

Similar as these two species are in the number of chromosomes, and the size relations of the chromosomes, it is striking to find the odd chromosome different in the growth stages. In *Philænus*, it is sometimes slightly oval in the earliest stages (Fig. 13), but more often round, and always round in the succeeding stages (Fig. 14, *X*). This is the condition in *Clastoptera*, *Aphrophora quadrangularis*, and *Aphrophora quadrinotata*. On the other hand, *Aphrophora spumaria* has a very long narrow dark-staining odd chromosome in the early growth stages (Fig. 30), which becomes round only in the stages just before the prophase of the first spermatocyte division (Figs. 31 and 32). Just such an odd chromosome was described by Dr. Stevens for the Harpswell form. But in *Aphrophora spumaria* there are no M-chromosomes. So there is variation in the Cercopidæ as to the shape of the odd chromosome in the growth stages.

Chromosomes and Somatic Variation.

The amount of variation in markings in *Philænus spumarius* made me suspect that they might be of more than one species. Some had distinct brown lines on the wings, forming a diamond, while others were of a light tan with only a dark streak at the posterior border. As one of the important cytological problems of the present day is to connect chromosomes with somatic variations, I made a careful study of the equatorial plates of different individuals with different markings. The plates of the first spermatocytes of seven differing individuals are shown in Figs. 1–7. There is evidently no clue to somatic variation here. The plates are strikingly alike, each has one largest chromosome

(*A*), and two almost as large (*B*, *C*), and most of them show a small odd chromosome (*X*) slightly to one side in the group. In Figs. 5 and 7, it is difficult to be certain which is the odd chromosome. Two somatic cells from two of these same individuals show also the same similarity in chromosomes, two longest and four almost as long (Figs. 20 and 21, *AA*, *BB*, *CC*).

The same sort of study in *Aphrophora spumaria* gave the same negative results. The somatic variation is great, but the chromosomes give no key to it. Fig. 26 is a plate from an individual with light spotted wings, Fig. 27 from one with light tan wings with no trace of spots, Fig. 28 one with dark spotted wings and black abdomen, and Fig. 29 one with streaked wings with the abdomen black on the sternites and yellow on the pleurites. All have one largest chromosome (*A*). There are probably two next largest as in *Philænus*, but that is no distinction between insects with different somatic characters. The only chance for chromosome variation among individuals lies in the peculiar small bodies shown in Figs. 34–37. These do not always stain as deeply as the chromosomes. They resemble somewhat the supernumeraries described by Stevens[1] in *Diabrotica*, and Wilson[2] in *Metapodius*, but they do not here occur condensed in the growth stages. There may be one, two, three, or four (Figs. 34–36). Whether this number is constant for each individual, I could not tell, as the testes from several individuals with similar wings and abdomen were preserved together. My material is so limited for the study of this point that I place these few drawings here in the hope that some one where the material is available will take the trouble to collect it and work out this point more thoroughly.

Summary.

1. The chromosomes of *Philænus spumarius* and *Aphrophora spumaria* were studied and compared with those of the four other species of Cercopidæ previously worked out. All have an odd chromosome which does not divide in the first spermato-

[1] Stevens, N. M., 1908, "The Chromosomes of *Diabrotica vittata*, *Diabrotica s oror*, and *Diabrotica 12-punctata*," *Jour. Exp. Zoöl.*, V., p. 453.

[2] Wilson, E. B., 1909, "Studies on Chromosomes," V., *Jour. Exp. Zoöl.*, VI., p. 147.

cyte division. The odd chromosome in the early growth stages of the spermatocytes varies in shape in the different species.

2. The individuality of certain chromosomes was traced in these two species of Cercopidæ. In both, the reduced number of chromosomes is 12. In *Philænus*, the identity of one large and two medium-sized chromosomes can be traced in the primary spermatocyte, secondary spermatocyte, spermatogonium, oögonium and male and female somatic cells. The odd chromosome is always one of the smallest. In *Aphrophora*, one largest chromosome always appears.

3. The somatic variation in both species is very marked, but there is no corresponding variation in the composition of the chromosome groups.

UNIVERSITY OF MAINE, ORONO,
November, 1912.

All figures were drawn with a camera lucida, 1/12 oil immersion and 12 compensating ocular, then reduced in the engraving to four fifths. The drawings of Plates I., II., and III. are all from acetocarmine preparations, but those of Plate IV. are from material fixed in Gilson and stained in iron hæmatoxylin.

EXPLANATION OF LETTERS.

A = the largest chromosome.

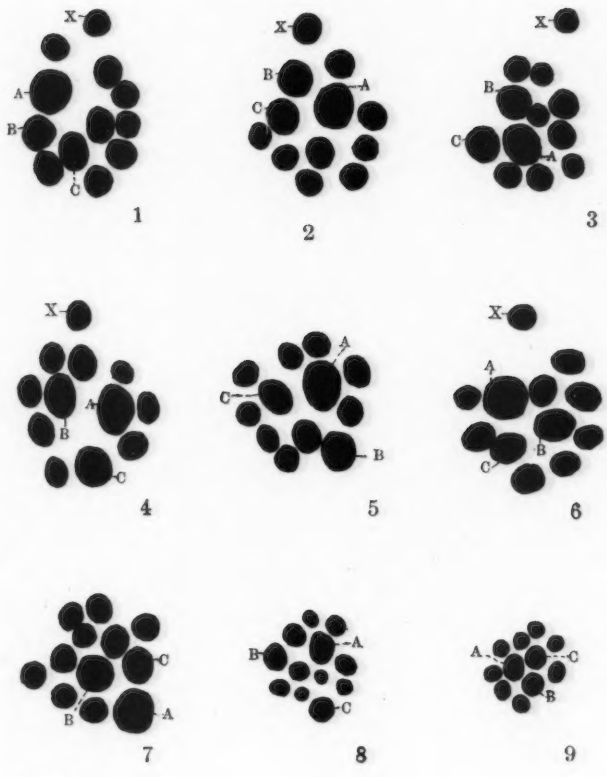
B, C = the two chromosomes almost as large as A.

X = the odd chromosome.

EXPLANATION OF PLATE I. (*Philænus spumarius*).

FIGS. 1–7. First spermatocyte metaphases from seven individuals with different somatic markings. All show 12 chromosomes, one largest (A) and two almost as large (B, C). Most of them show the odd chromosome at one side of the group (X).

FIGS. 8–9. Metaphases of the secondary spermatocytes, one with 12 and one with 11 chromosomes.



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EXPLANATION OF PLATE II. (*Philænus spumarius*).

FIGS. 10, 11. Two optical sections of one primary spermatocyte spindle such as is represented in Fig. 12, odd chromosome toward one pole.

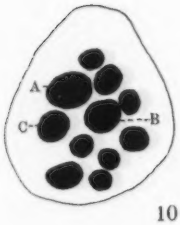
FIGS. 12, 16. Two metaphase spindles of the primary spermatocyte division.

FIGS. 13, 14. Early and late growth stages of the primary spermatocyte.

FIG. 15. Prophase of the primary spermatocyte, odd chromosome the only univalent one.

FIG. 17. Anaphase of the primary spermatocyte division, odd chromosome toward one pole.

FIG. 18. Two daughter plates of a primary spermatocyte division, each with 11 chromosomes, the odd chromosome between them.



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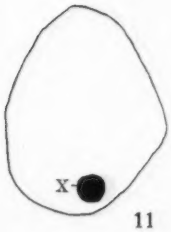

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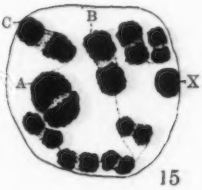

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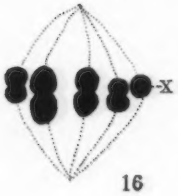

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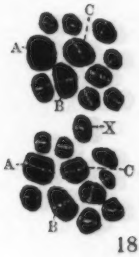

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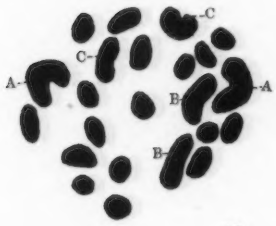
EXPLANATION OF PLATE III. (*Philænus spumarius*).

FIG. 19. Equatorial plate of a spermatogonium, 23 chromosomes, 2 largest (*AA*) and 4 next in size (*BB*, *CC*).

FIGS. 20–22. Male somatic cells, 23 chromosomes, size relations the same as in Fig. 19.

FIG. 23. Equatorial plate of an oögonium, 24 chromosomes, same size relations as in Fig. 19.

FIGS. 24, 25. Female somatic cells, 24 chromosomes, same size relations as in Fig. 19.



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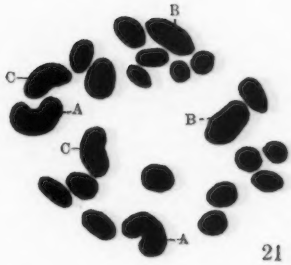

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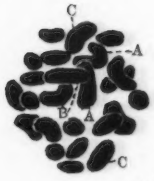

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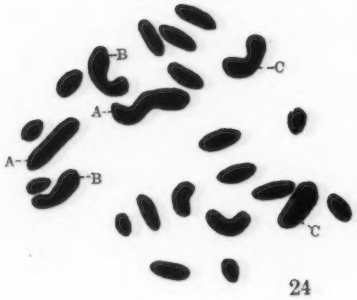

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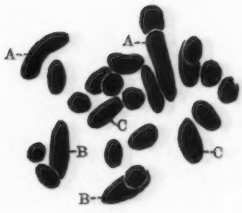


25

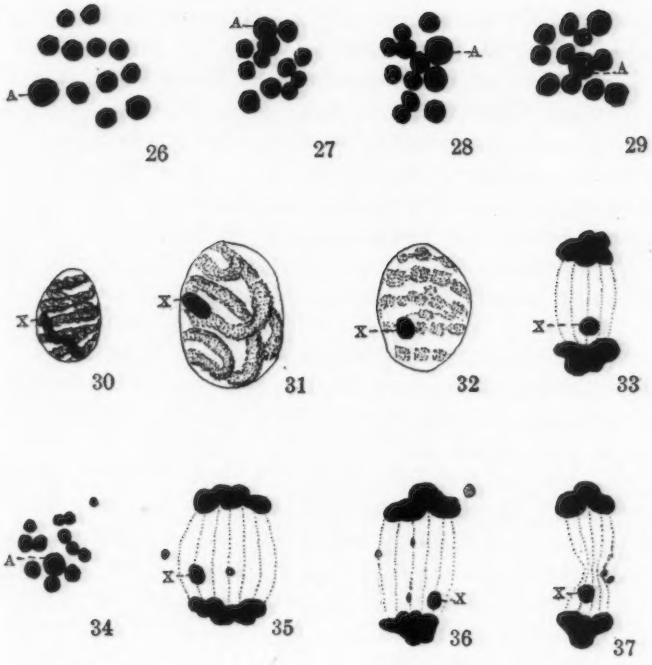
EXPLANATION OF PLATE IV. (*Aphrophora spumaria*).

FIGS. 26–29. Equatorial plates of primary spermatocytes of four different individuals with different somatic markings, one largest chromosome in each (*A*).

FIGS. 30–32. Growth stages of primary spermatocytes.

FIGS. 33, 35–37. Anaphases of primary spermatocytes, *X* toward one pole.

FIGS. 34–37. Drawings showing small chromatin bodies, which may be supernumeraries.



A. M. BORING.

BREEDING HABITS OF THE HETERONEREIS FORM OF NEREIS LIMBATA AT WOODS HOLE, MASS.

FRANK R. LILLIE AND E. E. JUST.

The senior author has used the eggs of the pelagic form of *Nereis limbata* in studies on fertilization for several years. During the seasons of 1911 and 1912 collections were made each night by the junior author, and records were kept which reveal considerable uniformity in swarming periods in accordance with the phases of the moon. In addition advantage was taken of the opportunity to study certain other features of breeding behavior. These observations are brought together in a paper partly for the sake of such general interest as they may possess and partly for the information of those who may have occasion to use this form for investigations.

The animals may be taken after sunset on certain nights, in general during the "dark of the moon," in the months of June, July, August and September. They appear swimming near the surface of the water very soon after sunset, and may be attracted by the light of a lantern and readily caught with a small hand net. The swarming usually begins with the appearance of a few males, readily distinguished by their bright red anterior segments and white sexual segments, darting rapidly through the water in curved paths in and out of the circle of light cast by the lantern. The much larger females then begin to appear, usually in smaller numbers, swimming laboriously through the water. Both sexes rapidly increase in numbers during the next fifteen minutes, and in the case of a large swarm there may be hundreds of males in sight at one time, though the number of females to be seen at one time rarely exceeds ten or a dozen.[1] In about 45 minutes the numbers begin to decrease and

[1] At the height of some runs, as for instance, on the evenings of June 12, July 5 to 8, and August 6–10, 1912 (see curves 6, 7 and 1), great numbers of females appeared. On one occasion at least a liter of females was secured in ten minutes, several being caught with each sweep of the net. Within the circle of the light on such nights as many as fifty females may be seen at once.

in an hour, or an hour and a half, all have disappeared for the night. Next night the scene may be repeated. The females are a fresh crop each night, as will be seen from the description below, though the same males may presumably appear on several successive nights, a circumstance which, if true, will partly explain the usual great numerical preponderance of males.[2]

In any given night the males invariably appear first. As the females appear in increasing numbers, the males gradually grow fewer. During the first nights of a run, as well as towards the end, the males are relatively more abundant. On nights of the greatest swarms, when the females appear in great numbers, the disproportion is perhaps not as noticeable. It seems, however, that there is no definite place in the run at which this sex ratio changes, although there are some nights throughout the summer when more females than males are caught.

During the light of the moon, with exceptions noted beyond, no *Nereis* are to be found. They therefore occur in four periods or "runs," during the summer, corresponding to the lunar cycles in the months of June, July, August and September. Each run begins near the time of the full moon, increases to a maximum during successive nights and sinks to a low point about the time of the third quarter, then again rises and falls to extinction shortly after the new moon. Each run is therefore typically divided in two sub-runs, or the curve of nightly numbers during a run is bimodal, with a deep depression about the time of the third quarter.

When a female appears she is soon surrounded by several males, if the latter are abundant, which swim rapidly in narrow circles about her. In a little while they begin to shed sperm, rendering the water milky, and soon she begins to shed her eggs, shrinking in bulk as she does so, until, a mere shadow of her former self, she sinks slowly in the water to die. We have never succeeded in keeping females alive for more than a few days after the eggs are shed.

[2] The worms are eaten voraciously by certain fish, as we ascertained in 1911 when *Nereis* were kept in aquaria with *Fundulus*. During August, 1911, it was often noted that fish attracted by the light seemed to keep down the number of *Nereis* caught. The slowly moving females were always more easily caught by the fish. This may therefore be another cause that influences the sex ratio.

If the animals are to be kept over night for work the next day, they should be placed in separate finger bowls, preferably one for each individual, but in any case never mixing the sexes. If then the bowls are placed in a cool place, as on a water table with water flowing around them, a considerable proportion of the females and all of the males will retain their sexual products over night, and the eggs may be fertilized as desired. One great advantage of *Nereis* for experimental work is that each swarming individual is always fully mature, and contains no immature sexual elements whatever.

I. The Causes of the Sperm-shedding and Egg-shedding Reflexes.

An incidental observation of the senior author put us on the track of the immediate cause of the sperm-shedding reflex. It should be noted that males retain the sperm very tenaciously as a rule, even after days in the laboratory. One day a male was dropped accidentally in a bowl of sea water which had previously contained a female. He immediately began to shed sperm, and swam round and round in the bowl very rapidly casting sperm until the entire 200 c.c. was opalescent. This suggested that the effect of the presence of the female upon the male was exerted not through sight or touch, but through some chemical stimulus, and a few simple experiments demonstrated this conclusively.

If a ripe female be kept in a finger bowl containing about 125 c.c. of sea-water for several hours, the water will usually be found to be charged with a substance which immediately incites the sperm-shedding reflex of the male. Thus on June 17, 1911, the following test was made with about 125 c.c. of sea-water in which a female had been kept over night. A small quantity of this charged sea-water was placed in a Syracuse watch crystal, and a male was dropped in (8:15 A.M.); at once he shed sperm. He was then returned to fresh sea-water and ceased shedding. At 10:30 he was again dropped into a watch crystal of the charged sea-water and again shed sperm; in a few seconds he was transferred to fresh sea-water, and he ceased shedding sperm. Again at 2:07 he was transferred to another supply of the charged sea-water, and again shed sperm. Returned to fresh sea-water

he ceased. At 2:20 he was tested again with positive results for the fourth time, and a little later was tested once more with water charged by another female and shed sperm once more.[1]

Thus each time that this animal was placed in sea-water that had contained a female for a certain length of time he responded instantaneously, and each time that he was removed and put in fresh sea-water he ceased to shed sperm.

This reaction is almost mechanical in its regularity, and can be secured with almost any male in good condition. Cases were found in which certain females apparently did not give off the effective substance, but they were very rare. As a general thing a large fully ripe female will so charge 125 c.c. of sea-water in an hour's time that any active mature male will give the sperm-shedding reflex almost instantaneously on being dropped in after the removal of the female, whereas even repeated transfers from one bowl of fresh sea-water to another causes no such response. If a male and female be placed together in a bowl of fresh sea-water they appear to stimulate one another very quickly, but it is usually several minutes, at least in the case of animals that have been kept in the laboratory over night, before the male begins to shed sperm; and the female never sheds her eggs until after the male has begun to shed sperm.

We have seen that some kind of emanation from the female incites the male to shed sperm. In the case of the female, however, it is not any comparable emanation from the male, but the presence of sperm in the sea-water that incites the shedding of eggs. The females are much more apt to shed their eggs spontaneously than the males to shed sperm. Apparently, when fully mature, many females are unable to hold the eggs back. But, in the case of those that do, it is impossible to cause the egg-shedding reflex by putting them in water that has contained males; on the other hand, the addition of a certain quantity of

[1] Mechanical shock, killing fluids, alcohol and fresh water, may cause the worms to shed their sexual products; probably any sufficiently strong shock may act similarly. But by slow addition of alcohol to the sea-water the animals may be stupefied and die extended without shedding the sexual products. Worms freshly caught react more surely to secretions of the female than those that have been in the laboratory over 18 hours. The reactions are not so definite in the late afternoon of the day after capture, and this is associated with a general lowering of tone.

sperm to the water containing the female will usually cause shedding of the eggs.

There is thus a very precise form of sexual behavior which was tested and demonstrated repeatedly during the seasons of 1911 and 1912. No attempt has been made to study the subject at all exhaustively as our attention was directed to other problems, but a few experiments were made dealing with the male reflex that throw some more light on the matter.

In the first place it is probable that the emanation of the female that incites the male to shed sperm comes mainly from the eggs. This could be shown in two ways: (1) Spent females, *i. e.*, those that have shed their eggs, do not produce the inciting substance in quantities comparable to the production of egg-bearing females. It was possible to obtain some effects by keeping four or five spent females in a small quantity of sea-water over night; but the effect on the male was so slight as probably to be attributable to the few remaining eggs in the female, or to egg secretions persisting in their bodies. Moreover mature males would not respond to the presence of immature atokous females. (2) The eggs alone produce the inciting substance quite rapidly; thus in one experiment about one third of the eggs of a single female charged about 10 c.c. of sea-water in 80 minutes so that a male shed sperm quite freely on being put in some of this water. Transferring to successive bowls of sea-water caused no reaction. The same male shed yet more freely on being transferred to the same quantity of sea-water from a bowl of 125 c.c. in which a mature female had stood over night. This was repeated with several variations always with positive results.

The tests showed that the eggs alone were considerably more effective than the spent females. The production of the stimulating substance should therefore be attributed mainly to the eggs.

The problem of the nature of the emanation that stimulates the males has received inadequate attention; but some experiments were made which narrow the field down considerably. In the first place the reaction is not due to the CO_2 excretion of the females. This follows from the fact that males do not produce any stimulating substance, though they excrete CO_2. A series

of tests was also made with various dilutions of sea-water supersaturated with CO_2, all to no effect.[1] In the next place it is not due to any substance common to the females of marine animals. Tests were made with sea-water charged in one case with the emanations of *Fundulus*, in another case with the emanations of another polychæte, *Chætopterus*. Males placed in portions of such charged sea-water gave no reaction whatever, but the same males placed in a portion of sea-water that had contained a female *Nereis* over night immediately shed sperm abundantly. The mature males of *Nereis limbata* will not react even to the ripe pelagic females of a nearly related form (*N. megalops*).

It is therefore probable that the substance in question is specific. In this there is involved no new biological principle. It is well known that the males of certain butterflies, for instance, are attracted even from long distances to the females by virtue of some air-borne emanation that is certainly specific. And the same principle must also be involved in the relations of males and females in many other instances.

Another property of the exciting substance is its lability. This is shown by its disappearance on standing in the course of about three days, and by its destruction by heat. As regards the first point, the following experiment may be noted: June 24, 1911. Four samples of sea-water charged by *Nereis* females were tested, each of which had been effective when fresh: *a* was 7 days old, *b* 5 days, *c* 3 days, *d* was fresh. Of these only *d* was effective in causing male *Nereis* to shed sperm. Each of the samples *a*, *b*, and *c*, had been tested previously at intervals and each was found gradually to lose its stimulating power in the course of about 3 days. As regards the heat-lability of the substance in question, a series of experiments have shown that it requires about 10 minutes boiling to destroy the power of an effective solution entirely. Five minutes boiling diminishes the effect considerably. The ineffectiveness after boiling is not due to the removal of oxygen, for it is impossible to restore its effectiveness by aerating.

The stimulating substance is either neutralized by the presence of sperm in the water owing to combination with some sperm

[1] See footnote, p. 150.

substance, or it ceases to be effective in the presence of a certain concentration of sperm. This is shown by the following experiment, June 20, 1911: A male put in about 8 c.c. of the water charged by a female immediately shed sperm which was stirred up by his movements; in a little while he stopped shedding. He was then transferred directly to another equal amount of the original charged water and the performance was repeated. A third transfer gave the same result, and on the fourth transfer a small amount of sperm only was shed, the male being practically spent by this time.

The same phenomenon was observed on other occasions. The explanation is, I believe, that the stimulating substance is a sperm agglutinin produced by the eggs, which enters into combination with the spermatozoa and thus disappears from the solution in the presence of a sufficient quantity of sperm. The reasons for this opinion are: (1) The sea-water charged by the female does, as a matter of fact, contain a sperm agglutinin which can be shown to be neutralized by the addition of a sufficient quantity of sperm. (2) This sperm agglutinin possesses about the same degree of heat lability as the substance effective in causing the sperm-shedding reaction of the male. The relations of the sperm to egg-secretions will form the topic of another paper, but they are summarized in a preliminary paper by the senior author in *Science*, N. S., Vol. XXXVI., p. 527. The conclusion to which we have come is, therefore, that the same egg-secretion of *Nereis* which agglutinates the spermatozoa is the effective stimulus in the sperm-shedding reflex of the male. This is the simplest explanation of the facts and may be held until some reason is shown for believing that different substances are involved. Such a condition is particularly well adapted to secure fertilization under the conditions of the breeding habits of *Nereis*.

II. Data on the "Runs" and the "Swarms."

By a "run" we mean an entire lunar cycle of the pelagic "*Heteronereis*"; by a "swarm" the nightly occurrence. A general statement of the facts was given in the introduction. The precise data with some comparisons follow:

1. *The Swarms.*—The swarms consist only of fully mature males and females; indeed we never secured even a single swarming animal which was not fully mature, or which contained any immature sexual elements mingled with the mature ones. The occurrence of swarming is dependent more upon the lunar cycle than any other factor; the relations with reference thereto are given under the second heading, "*The Runs.*" The second most important factor in swarming is time of day; swarming begins invariably soon after twilight and continues for an hour or two at the most. A third factor is weather; very bad weather with heavy wind and rain may prevent swarming, or at least prevent the animals from coming near enough to the surface to be seen. A fourth factor is light, partially included, of course, under the second factor. But we have reference here especially to conditions that occur at the time of full moon or a little later, when swarming may begin after twilight, and be suddenly cut short by the appearance of the moon above the eastern hills.[1] On the other hand during the light of the moon, *i. e.*, from shortly after new moon until near the time of the full moon the animals do not swarm even if the moon is entirely concealed by thick clouds.[2] The stage of the tide whether high or low was not an observable factor at our station, where the range of tidal movement is only about 18 inches. Neither do the summer variations in temperature appear to be an important factor; in any event the temperature of the water varies but little during the swarming season.

We have gained the impression that all the absolutely mature animals of any given locality swarm as soon as the right conditions are offered. This would involve the assumption that the maturing of the animals is dependent on some relation of the life history to the phases of the moon, involving probably,

[1] An exception to this was noted at the time of full moon in July, 1912, when after moonrise both males and females were abundant. (See curve 1, July 28 and 29.)

[2] As an exception it may be noted that the end of the June run (1912) extended to within three days of the full moon (see curve 6). But the general rule is as stated. Special attention was directed to this point both in 1911 and 1912. There were several cloudy nights during the light of the moon in these years about the time of the first quarter of the moon; for instance, on July 21, 1912, a hard rain fell in the afternoon and the evening was dark and cloudy, but no worms could be found. We often tried collecting in dark places under wharves, etc., on moonlit nights, but always without success.

through lunar tidal variations, rhythmical alterations of conditions of nutrition. (Cf. Hempelmann, 1911.) This assumption at least explains fairly well the conditions as we find them.

2. *The Runs.*—The collector receives very definite impression concerning the size and composition of swarms and the general features of the runs. But to give these quantitative expression is not a simple matter, though it is essential in order to reveal the order that exists. Moreover, any quantitative system of representing the data must be based on some one feature to the exclusion of others, and it is of course limited to this extent. As a result of experience we finally decided in the first place to limit the collections used in the tables to one station, and in the second place to record only females caught. The latter provision is due to the relatively enormous number of the males on certain nights which makes accurate estimation out of the question. The collecting was done each evening from the same station in the same way as nearly as possible. Practically complete records were kept for 1911 and 1912, and these have been tabulated in the form of curves in which the ordinates represent the number of females caught and the abscissæ represent days from the first quarter of the moon.

Of these records of runs we may select that of August, 1912, as being in some respects the most typical (see curve 1). It begins on the fifth day after the first quarter of the moon (July 25), and is quite atypical in this one respect, for no other run began earlier than the eighth day. On the fifth day two feamles were caught, the sixth day one, seventh day ten, eighth day eight, then the catch gradually ascends to a maximum on the twelfth day. The numbers in the successive swarms then rapidly diminish to the 15th day, when only three females were caught, but on the 16th day there were sixteen females, and on the 17th, 18th, 19th, 20th, and 21st, over thirty. The numbers then gradually decreased to the 26th day, which was the last day of the run. This run had been preceded by eight days in which no *Nereis* occurred, and it was followed by a barren period of eleven days It was therefore sharply separated from preceding and succeeding runs.

The other runs may be compared with this one as follows:

1. June, 1911; curve 2. The first collection was made on June 12, one day after the full moon or ten days after the first quarter. Judging by experience this was probably the second or third day of the run. That night 12 females were caught. The curve of this run is bimodal, sinking to zero on the 20th day from the first quarter, and then rising again. The last swarm of the run was on the 28th day after the first quarter. There is a depression in the first mode due to some unknown cause. This run was separated from the following July run by eleven days in which neither sex could be found.

2. The July, 1911, run (curve 3) began on the ninth day after the first quarter of the moon, thus on the night of the full moon (July 11). The curve of this run is also bimodal with a deep depression from the 17th to the 21st days; the last swarm was on the 26th day. Here also there is a depression in the first mode. There was an interval of eleven days from the last swarm of the July run to the first August swarm.

3. August, 1911. The run (curve 4) began on the eighth day after the first quarter and lasted to the 26th day. While the curve is decidedly irregular it is nevertheless distinctly bimodal, with a depression to zero on the 17th day. Seventeen days elapsed before the beginning of the September run.

4. September, 1911. The run (curve 5) began on the thirteenth day after the first quarter. The swarms were extremely small and few in number. But parts of the two modes of the usual curve may be recognized.

5. June, 1912; curve 6. The first worms were secured on the 13th day after the first quarter of the moon. I regard the collections from the 13th to the 17th days as constituting the first mode of the curve. The second mode is very unusual, first in its irregularity, and second in its duration; it continued in fact ten days after the new moon, and it exceeds all other records in this respect by six days. There was thus an interval of only four days between the end of June and the beginning of the July run in 1912. The last seven days of the June run thus occurred during the "light of the moon," a circumstance which must be regarded as significant. Swarming can thus take place during moonlight. Its usual absence must therefore be inter-

preted to mean that completely metamorphosed animals usually do not occur during this time.

6. July, 1912; curve 7. The run began on the ninth day after the first quarter of the moon, on the night of the full moon (June 29). This run shows no evidence of two modes, for the lack of animals on the second night of the run cannot be interpreted as the intermodal depression. The run continued until the 27th day. The absence of two modes in this case may be attributed to overlapping of the two sub-runs making up each run. This run was followed by eight days without any swarms. The August, 1912, run, already described, finished on the 26th day after the first quarter; and there was an interval of eleven days before the beginning of the September run.

7. September, 1912; curve 8. This run began on the eighth day after the first quarter of the moon, and ended unusually early on the 23d day. Though the curve is irregular in its first part there is some evidence of bimodality.

The two features that stand out most prominently in these records are the relation of the runs to the lunar cycles, and the double character of each run. The following tabulation brings out the first point; the days being numbered from the first quarter of the moon in each case:

1911. First run, tenth to twenty-eighth day.
Second run, ninth to twenty-sixth day.
Third run, eighth to twenty-sixth day.
Fourth run, thirteenth to twenty-first day.

1912. First run, thirteenth to thirty-fourth day.
Second run, ninth to twenty-seventh day.
Third run, fifth to twenty-sixth day.
Fourth run, eighth to twenty-third day.

As regards the second point, the curves show sufficiently well that each run is made up of two sub-runs. But they are not usually sharply separated by an interval as in the case of the main runs; however, the general rule is that the interval occurs near the time of the third quarter of the moon. Thus the low points in the runs as tabulated are:

1911.	Days after First Quarter.
First run	20
Second run	17–21
Third run	17
Fourth run	15–21

1912.	
First run	16–17
Second run	?
Third run	15
Fourth run	15

The third quarter of the moon comes 16–17 days after the first quarter.

Hempelmann (1911) finds that the swarms of *Nereis dumerilii* at Naples tend to center around the time of the first and third quarters of the moon; none were caught on the days of the new moon or the full moon. Thus, the monthly curve is bimodal as in the case of *Nereis limbata*, but the modes, occurring at the time of the first and third quarters, do not coincide, but alternate with those of *N. limbata* which approximate the times of the full moon and the new moon. The animals were secured from the daily plankton catch taken in the early morning hours, so little is known about the duration of the swarming or the time of its maximum. But the indications are that the time of the daily swarm is early morning (before sunrise?), and this furnishes another contrast to *N. limbata*. The runs of *N. dumerilii* occurred at Naples from October to May.

According to Akira Izuka (1903) the Japanese palolo (*Ceratocephale Osawai*) swarms closely following the new moon and the full moon. The swarms occur shortly after sunset and last an hour and a half to two hours. Each run is limited to three or four nights in the months of October and November. Thus there are two entirely distinct runs each month in this case corresponding to the sub-runs in the case of *Nereis limbata*. About ten days after each swarming period transitional half-epitokous forms are found in the bottom mud, which finish their metamorphosis in time for the next swarming period, after which only immature animals are to be found in the mud. The periodic swarming is thus dependent on periodic maturation of the animals. In many respects the swarming of *Ceratocephale* is more like that of the Woods Hole *Nereis* than any other form.

The breeding habits of the Pacific palolo (*Eunice viridis*), the Atlantic palolo (*Eunice furcata*), and *Odontosyllis* (Galloway and Welch, 1911), are similar with respect to periodicity dependent on lunar phases, though of course different in many details.

Some other annelids (*e. g., Amphitrite;* see Scott, 1909) exhibit a lunar periodicity in breeding habits. The periodicity in *Nereis limbata* thus belongs to a large class of phenomena, which includes breeding seasons of some plants as well as many other animals, for which no adequate general cause has yet been found.

III. The Life History of *Nereis limbata.*

This subject is still under investigation. It is obvious that interpretation of the swarming behavior must wait for fairly complete knowledge of the life history. The present indications are that the worms either do not mature in the atokous condition, or, if they do so, that it is at an earlier time of year than our collections were made. The atokous forms were secured both in scrapings from piles of wharves and also by digging, and were kept under observation in the laboratory. All that matured underwent metamorphosis to the epitokous condition. Despite repeated efforts we failed to rear mature worms from artificially fertilized eggs. However, a few were raised to young worms about 5 mm. in length, about eight weeks after fertilization; but all of them died after an attempted change in culture methods.

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GENERAL DESCRIPTION OF CURVES.

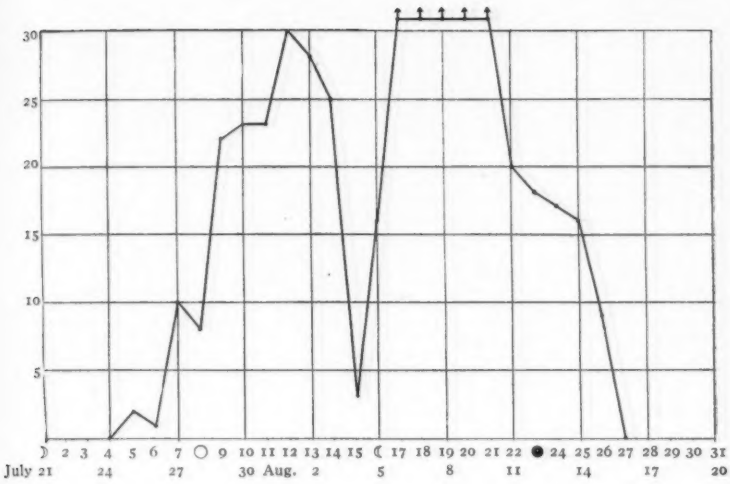
The ordinates represent the number of females caught.

The abscissæ represent days from the first quarter of the moon. The various phases of the moon are indicated. The days of the month are given in the second horizontal row of figures below the curves.

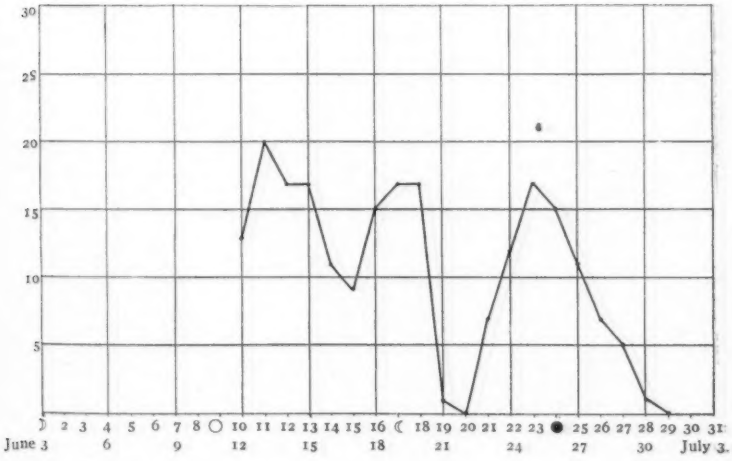
CURVE 1. The August run, 1912.

CURVE 2. The June run, 1911.

CURVE 1

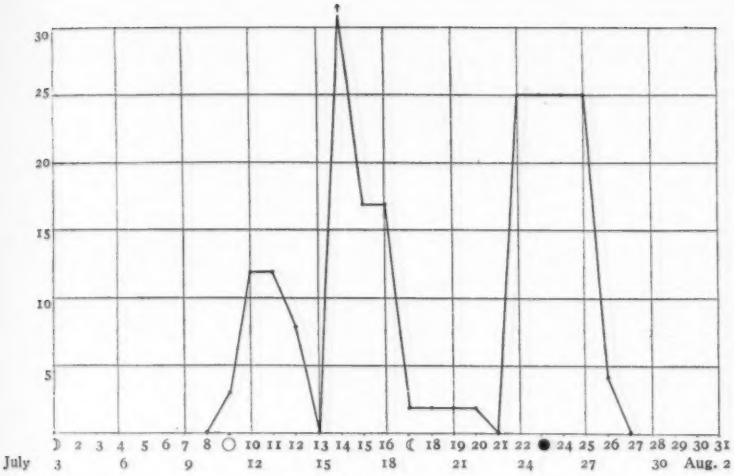


CURVE 2

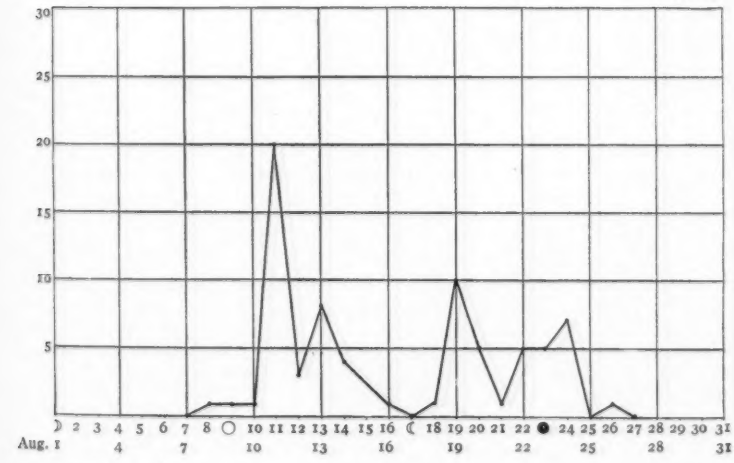


CURVE 3. The July run, 1911.
CURVE 4. The August run, 1911.

CURVE 3



CURVE 4

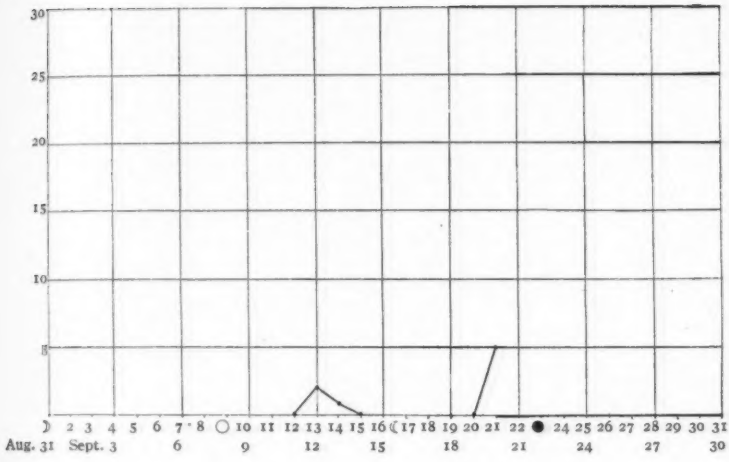


LILLIE AND JUST

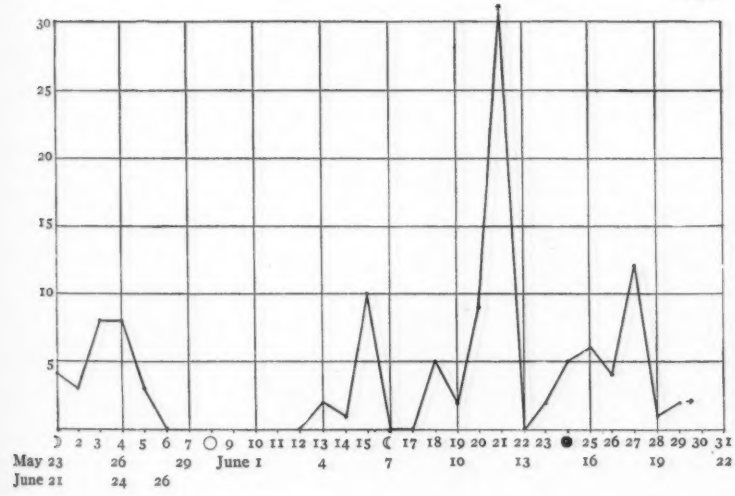
CURVE 5. The September run, 1911.

CURVE 6. The June run, 1912. The part of the curve to the left is the end of the June run as indicated by the arrow at the extreme right, and by the second row of dates which refer to this part of the curve (see footnote, p. 154).

CURVE 5

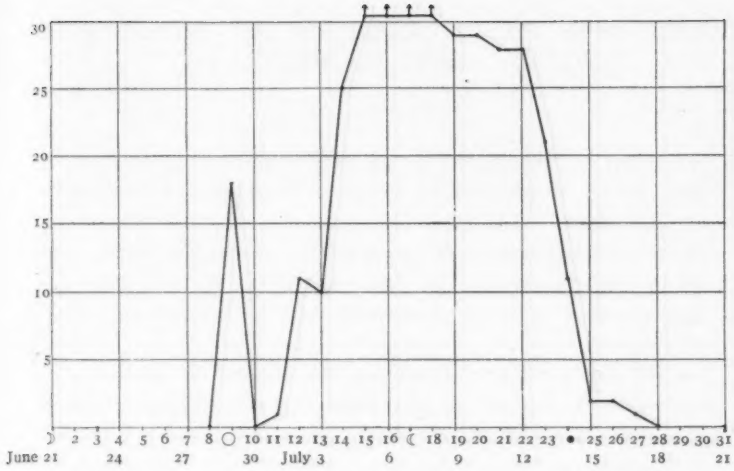


CURVE 6

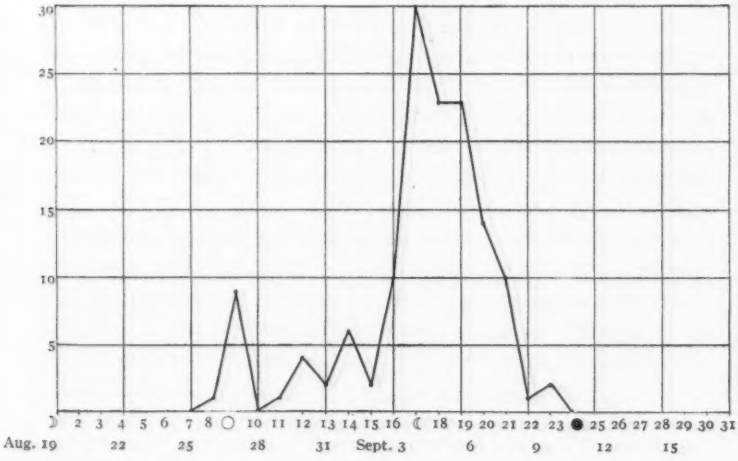


CURVE 7. The July run, 1912.
CURVE 8. The September run, 1912.

CURVE 7



CURVE 8



THE EFFECT OF DISTILLED WATER UPON THE FIDDLER CRAB.[1]

J. F. ABBOTT.

At present, *Fundulus* offers the only exception[2] to the statement that the unprotected epithelial surfaces of marine and fresh-water animals are permeable to distilled water and to electrolytes in solution. In all cases life appears to be preserved by some sort of an "adaptation" as yet unexplained which limits the permeability or controls it so as to maintain the integrity of the body fluids. Sumner[3] has shown that the gills in a variety of Teleosts are permeable, and Scott and White[4] have conclusively shown the same thing in *Mustelus*, while Garrey[5] and others have demonstrated the fact in a great number of invertebrates. The apparent exception offered by *Fundulus* is therefore the more remarkable.

While working at the Beaufort Laboratory of the U. S. Fisheries Bureau my friend Professor W. E. Garrey called my attention to the fact that the fiddler crabs (*Uca pugillator*) which abound in that region were passing indifferently from the sea to the overflow of an artesian well and he suggested that this form, like *Fundulus*, might offer an exception to the statement just made regarding the permeability of membranes. A few tests seemed to confirm this, for the crabs appeared to live indefinitely in pure distilled water as well as in salt solutions of various osmotic densities. The writer therefore devoted the greater part of the past summer to a study of the reactions of this animal to various salt solutions. For the privilege of working at the Beaufort Laboratory he is indebted to the kindness of the Hon. George M. Bowers, U. S. Commissioner of Fisheries, and to Mr. Lewis Radcliffe, the

[1] From the Laboratory of the U. S. Bureau of Fisheries at Beaufort and the Zoölogical Laboratory of Washington University.

[2] Species of *Cyprinodon* and *Gambusia* apparently behave in the same way, although the facts have not been thoroughly worked out.

[3] U. S. B. F. Bull., 1905, Vol. 25, p. 97.

[4] *Science*, N. S., 1910, Vol. 32, p. 767.

[5] *Biol. Bull.*, 1905, Vol. 8, p. 257.

director of the laboratory, he is under especial obligation for the constant courtesy and generous assistance of which he was at all times the recipient. The present paper deals only with the effect of pure distilled water upon the fiddler crab.

The crabs inhabit burrows in the sandy or marshy shores, the openings of which are usually, though not always, covered by the tide. At high tide the crabs retreat to their holes which they plug up with sand. As the tide ebbs they emerge and begin feeding, frequently going a considerable distance from their holes. In the interval between high tides, if the sun is bright and the day hot the males go through characteristic and energetic "dances." Altogether they spend many hours of vigorous activity out of the water. A related genus of the same family, *Ocypoda*, lives out of the water almost entirely and spends most of its time running about on the dry sand.[1] In accordance with this habit a peculiar modification has developed in connection with the gill chambers. In these two genera these are very large and cavernous, the two chambers together equalling approximately the space occupied by the viscera. The gills lie in the floor and the large space above them is normally filled with liquid. Communicating with this chamber, in addition to the ordinary openings for the exit and entrance of water, there is an aperture between the basal joints of the third and fourth legs, bounded by chitinized lips which bear tufts of hairs. This opening communicates between the cavity above the gills and the exterior by a sort of canal provided with a ciliated valve. By means of this arrangement, not only is the animal able to store up a large amount of water above the gills but it is also possible for a constant gaseous exchange to take place between this contained water and the air without. In other words the crab when out of the water is able to breathe air.

This accounts for the apparent tolerance of the animal to pure distilled water. When immersed in distilled water it feeds out through this channel some of the sea water contained in its gill chambers. As with some other crustacea a very small amount of NaCl in the surrounding medium is sufficient to preserve the integrity of the membranes and permit the animal

[1] Cf. Cowles. Carnegie Inst. Papers, Tortugas Lab., II., 1908.

to live in spite of the greatly altered osmotic pressure. That the crab accommodates itself in this way was shown by the following experiment. Five crabs that had been running about in a dry tank for a week were rinsed off in running fresh water, dried, and immersed in 250 c.c. of pure distilled water which was removed and renewed at short intervals. These samples were then titrated for chlorides by the Volhard method with the following results:[1]

Interval of Immersion in Pure Distilled Water.	Cl in Milligrams.
30 minutes	0.038
1 hour	0.188
2 hours	0.263
3 hours	0.132
3½ hours	0.297
Total Cl	0.918

The crabs were moribund in another half hour. The gill chamber capacity of each crab was roughly ½ c.c. The percentage of Cl in Beaufort sea water is 1.963 gm. per 100 or .049 gm. to 2½ c.c. The detectible salinity emitted by the crabs was thus only about 20 per cent. of that of sea water. Some may have been "held back," but it is more probable not only that the crabs in the experiment, which had been out of water for a week, but also crabs running about out of the water normally have their gills bathed by a liquid of only about one fifth the concentration of sea water. Experiment has shown that they will live indefinitely when immersed in very much greater dilutions, although I have not found that they will endure such a dilution as that used by Bullot[2] for fresh-water *Gammarus* (0.00008N).

In subsequent experiments the side of the gill chamber was cut away and the contents rinsed out with distilled water so that not only were all salts washed away but the gills were directly exposed to the action of the medium. Even under such circumstances the crab does not at once die when placed in distilled water but will live actively usually from four to six

[1] For assistance in carrying out this portion of the experiments as well as for many helpful suggestions the writer is indebted to Mr. Wm. J. Crozier.

[2] Univ. of Calif. Pub. Physiol., 1904, Vol. I., p. 199.

hours. Death appears to be due to a loss of necessary salts by diffusion out of the body fluids together with a disturbance of vital equilibrium on account of the absorption of water. In most marine invertebrates death very quickly follows a transfer into fresh water, that is, the limiting membranes are absolutely permeable to water and osmotic diffusion is very rapid. (The body fluids of all marine invertebrates, so far as known, are isosmotic with sea water.) In *Uca*, on the other hand, the gill membranes are apparently impermeable to water until the strongly solvent power of the distilled water has attacked or partially dissoved them and thus rendered them partially permeable. That they are eventually rendered permeable is shown in the following experiments. A larger species of *Uca* (*U. minax*) was chosen because of its greater size, but the habits and reactions of the two species are identical. The carapace of each crab was cut away on both sides to expose the gills, and the gill chamber was rinsed out. The carapace was then thoroughly dried with absorbent cotton and the crab was carefully weighed, and immersed in 200 c.c. of water distilled in Jena glass over potassium dichromate and sulphuric acid. At intervals this water was changed for a fresh amount and the sample titrated for chlorides with potassium thiocyanate against silver nitrate, using iron alum as an indicator. The following results were obtained:

TABLE I.

I.	II.	III.	IV.	V.	Chlorides (in Mg.) After
0.205					1 hour.
	0.135		7.420	5.312	3 hours.
5.600	9.431	26.09			5 hours.
9.210		1.700	(died)	0.954	7 hours.
					9 hours.
		8.91			11 hours.
15.016	9.516	36.705	(7.42)	6.266	Total chlorides.
7.588	3.544	8.463	6.011	4.638	Original wt. in grams.
7.813		8.460	6.599	4.833	Final weight.
0.225	0.677	0.005	0.588	0.195	Gain in wt.

REMARKS.

Crab No. 1 died shortly after 7 hours.

Crab No. 2 died after 4 hrs., weighed immediately. Big claw cast at beginning, wound seared with nitric acid.

Crab No. 4 died shortly after 7th hr.; final chloride determination not taken.
Crab No. 5 died shortly after 7th hour.

It will be seen that without exception all the crabs gained in weight through immersion in distilled water. Likewise all the crabs lost chlorine, presumably through diffusion. There is no doubt that some of this loss may have been occasioned by bleeding, yet the outer shell of the gill chamber is very little vascular and such a loss must be inconsiderable. It, however, might be sufficient to account for the irregularities in the amounts of chlorine lost by the various crabs. An exception must be made of Crab No. 3 of which the branchiostegite was cut away very deeply and bled into the water. This accounts for the very small gain in weight and exceptionally high chlorine loss. Yet this individual for some reason not evident lived longer than any of the others.

If death is brought about by the extraction of necessary salts from the organism it ought to make considerable difference whether the amount of water that surrounds the animal is great or small. For we have seen that the presence of a very small amount of salt in the water (such as exists in ordinary well water) is sufficient to preserve the membrane or at least to greatly retard its solution by the water. If the amount of water were scant the crabs might be able, by losing a small amount of salts to it and thus raising the osmotic density of the medium ever so slightly, yet thereby to retard the solvent effect of the distilled water on the gill membranes. To test this, fifty tall, wide mouth bottles were arranged, in each ten of which was put, respectively, 50 c.c., 100 c.c., 150 c.c., 200 c.c., and 250 c.c. of distilled water. In each bottle was placed a crab (*U. pugillator*). The gill chambers were not opened but the lot were subjected to running fresh water for fifteen minutes, a procedure which experience has shown displaces practically all the salt water that may be retained in the gill chamber. The results are tabulated below, the figures representing the number of crabs alive at the different intervals of time.

TABLE II.

	50 c.c.	100 c.c.	150 c.c.	200 c.c.	250 c.c.
4 hours	10	10	10	10	10
8 hours	10	10	9	9	8
12 hours	10	10	8	6	2
18 hours	10	7	5	4	0
22 hours	10	1	1	0	
24 hours	9	1	0		
48 hours	6	0			
72 hours	2				

SUMMARY.

1. The fiddler crabs of the genus *Uca* are able to endure abrupt and profound changes in the osmotic density of the water which they enter, passing from sea water into ordinary "fresh" water with impunity.

2. They are able to spend long periods out of the water by the aid of a mechanism by means of which they store up a quantity of water above the gills and aerate it directly by contact with the air.

3. Their gill membranes are only relatively not absolutely impermeable. In the presence of pure distilled water they both lose salts (as indicated by the titratable chlorine) and also gain weight through the absorption of water.

4. They will live a much longer time in small quantities of distilled water than in larger quantities. This is probably due to the fact that the salts diffused out from the tissues although small in amount are sufficient, in a small quantity of water, to raise the osmotic tension of the latter sufficiently to inhibit its solvent action on the gill membranes and thus to retard the further diffusion of salts. In a larger quantity of water this point cannot be reached before the debilitating effect of the loss of salts results in the death of the crabs.

ON THE ORIGIN OF DOUBLE-YOLKED EGGS.

OTTO GLASER.

Introduction.

By comparing five cases of double eggs that happened to fall into his hands, with a considerable number described in the literature, Parker ('06) was able to divide these abnormalities, on the basis of the factors probably involved in their production, into three classes: "First, those whose yolks have come from an abnormal ovary, but have passed through a normal oviduct; secondly, those whose yolks have come from a normal ovary, but have passed through an abnormal oviduct; and finally those produced by an ovary and oviduct, both of which have been abnormal in their action" (p. 17).

To the first group belong eggs which contain ordinarily two yolks, surrounded either by individual vitelline membranes or by a common one. The second class is made up of eggs in which normal yolks are imbedded in abnormal secondary envelopes, whereas the third is composed of cases in which one egg, consisting usually of shell, shell-membranes, albumen and a small yolk, is enclosed in an outer one of normal construction. Nine cases found in the literature by Parker, four additional ones studied by himself, as well as some of those referred to by Hargitt in a neglected paper ('99) and in a later one ('12), belong to this group.

In this article I shall report observations on a case belonging to the first class, and I do this, not because double-yolked eggs are sufficiently rare to warrant further description, but because I have been unable to find in the literature accessible to me any account of the ovarian abnormalities associated with the production of this type of egg. In fact from Parker (*loc. cit.*, p. 16) one gains the impression that these peculiarities are hardly marked enough to justify the use of the word "abnormal." Thus he says: "The laying of eggs with two yolks may become, as Landois ('78, p. 24) declares, almost habitual with certain

hens. Bartels ('95, p. 143) states that the hen that laid the double egg described by him had often laid such eggs, and Immermann ('99, p. 8) records the case of a hen that laid such an egg about every eight days. Apparently this is as much an organic peculiarity of certain hens as is the production of twins by certain individuals in the human species, and while it may be called abnormal in that it is unusual, it is in no sense indicative of serious organic derangement or disease."

Despite the fact that the origin of double-yolked eggs can be attributed, as it is by Parker, to an ovarian peculiarity, such as the simultaneous discharge of two yolks from separate follicles, or the rupture of one follicle containing two yolks, there nevertheless are two other possibilities. In the first place, an ovum discharged into the infundibulum might fail to be moved downward by peristalsis until the ovary had discharged a second time, in which case we might be dealing either with a deficiency of substances normally inducing these movements, or with subnormal irritability on the part of the oviduct. In the second place, the "organic peculiarity of certain hens" may have an ovarian basis decidedly "indicative of serious organic derangement" if not of disease. The ovary which I have studied is certainly pathological although I cannot conclude that the conditions found are the only ones that induce double-yolked eggs, or that they always do so.

Unfortunately the bird on which my data are based died during my absence and the oviduct was not preserved. However the ovary seems to me capable of explaining why she laid abnormal eggs, although this does not show that the oviduct was normal either in structure or in action. Normality however seems likely, for if there had been peristaltic difficulties it seems reasonable to suppose that occasionally at least an egg abnormal in other respects would have been produced. This is not known to have happened in the five years during which this hen was under fairly constant supervision.

Clinical History of the Individual.

The bird in question, a white leghorn belonging to Mrs. Wm. Looker, of Ann Arbor, was hatched in the spring of 1906 and lived

until June, 1911. She was a beautiful example of the breed, but had an unusually large comb. She was a loud and boisterous cackler, and always made a great fuss after laying. Apparently she never exhibited any tendency to set. Although it cannot be stated with certainty that she never laid normal eggs, it is certain that a very large percentage was abnormal.

The eggs during her last year measured on an average 7.5 cm. in their long diameter, 4.5 cm. in their short. The yolks, always in individual vitelline membranes, were equal in size with an average diameter of 3 cm. They were always in contact, or practically so, and were surrounded by a common jelly mass.

The records are too meager to show whether there was any rhythm in her laying. On occasion she laid double-yolked eggs on two or even three successive days. Spells of hyperactivity such as these were frequently followed by periods of indolence lasting from one to several weeks. Sometimes she would lay every other day for a period.

Although the eggs were always large they decreased in later years. Even then laying caused difficulties as evidenced by noises suggestive of struggle. After a period of laying the bird nearly always "moped," hanging her head and refusing to eat. These sick spells were not noticed during the first year or two, although there was never any eagerness for food.

I have given these details because they may be valuable as clinical symptoms, and because they show that the ovary, which, as we shall see, was abnormal structurally, was so physiologically as well. In fact I am inclined to think that the structural peculiarities of this organ are a consequence primarily of some physiological defect, and that the abnormal spatial relations brought about in this way gave the physical basis for the production of double-yolked eggs.

The Anatomy of the Ovary.

The ovary was removed immediately after the death of the bird and fixed in Zenker's fluid by my brother, R. W. Glaser. The gross appearance as well as numerous details are reproduced in Fig. 1, which I made by first printing an outline of the organ through glass on blue-print paper, and after transferring this to bristle board, filling in the necessary minutiæ freehand.

Even a casual glance shows that this organ is markedly abnormal. Most striking of all is the presence of many medium-sized follicles which, instead of being held to the main mass of the ovary by relatively short, stout necks of tissue, are attached

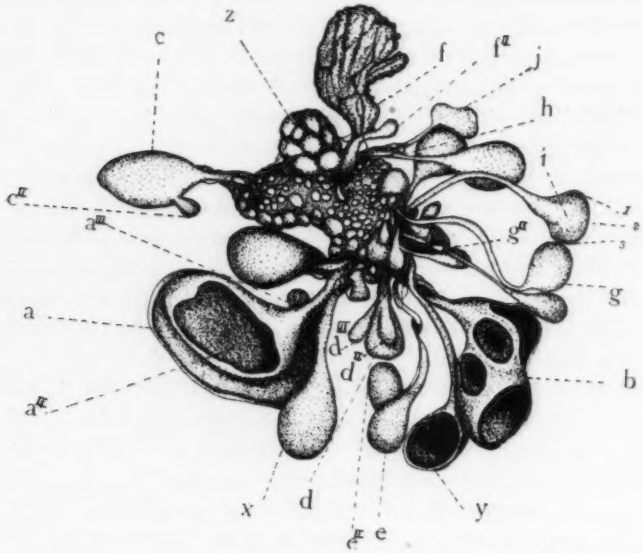


FIG. 1. Abnormal ovary of hen that laid double-yolked eggs. Description in text, p. 178.

by suspensoria more or less twisted and at times over 3.5 cm. in length. Originally these appendages formed a complicated snarl. To facilitate both drawing and description they were carefully dissected apart as in the figure.

Four other features, some of them equally striking, are worthy of note. First are the compound follicles, *a* and *b;* second, apparently budding follicles at *c*, *d*, and *e;* third, apparently branching suspensoria, *f*, *g*, and *h;* and finally a considerable number of fine threads running at various angles from one suspensorium, follicle, or portion of the central mass, to another.

The origin of these various structures can be inferred with reasonable certainty in most cases. As far as the nature of the suspensoria is concerned, I think there can be little doubt,

although there are at least two possibilities as to the mechanism of their development. Sections show that they are composed entirely of connective tissue, and that they may or may not possess a lumen. Blood vessels are abundant. From these facts, as well as from the total absence of granulosa cells, I conclude that the suspensoria are not modified follicles, but greatly elongated projections of the ovarian surface, tunica albuginea, filled with stroma.[1]

I am not certain how these elongations have arisen. It is conceivable that they are the products of abnormal growth; it is equally conceivable that they are the result of stretching, due to weakness or readiness to flow on the part of the tunica albuginea. In a bird in its normal upright position, the sudden development (Riddle, '11) of a large yolk mass in a given follicle must seriously increase the load sustained by the tunica, and if this for any reason is weak, stretching in these regions might easily result. It is interesting in this connection that all of the follicles with long suspensoria are either well along in their growth periods, or are attached to those that are. Comparative study of the compound follicles, of the branching suspensoria, and of the threads makes me incline definitely toward the second hypothesis.

Such study not only suggests that all the structures mentioned are the outcome of one and the same process, but also how the more complicated follicles may have originated. *B* is a good one to begin with. Before dissection the surface of this irregular follicle gave but faint indication of the four completely separate cavities which it contains. Sections of the interfollicular walls show one continuous connective tissue mass bridging the distance from one granulosa to the other. This latter membrane in all the follicles sectioned appears much hypertrophied. It is at least four times thicker than normal and contains quite large intercellular spaces or lacunæ.

How such a complex follicle could have originated can be

[1] This naturally suggests the question whether the enlargements at their distal ends are really follicles, or merely abnormal structures superficially resembling them. The discovery of large yolks with smooth surfaces within these bodies, as well as the presence of fibrosæ and granulosæ all in proper relation, leaves no doubt that we are dealing with genuine egg follicles.

answered by studying the others. Follicle *c* suggests that compound follicles might have originated by a process of budding. To test this view part of the wall of *c* together with the attached follicle *c''* was cut into sections 10μ thick. To my surprise no connection whatever between the lumina of the two follicles could be found. The place where this was expected showed the granulosa and fibrosa of both, and between the fibrosæ, a thick connective tissue mass highly vascularized. I examined other apparently branching follicles, such as *e*, and again found the same thing true. In certain other instances connections between adjacent follicles do exist, but these are not via the necks of the attached follicles, but through their sides, and must therefore have come about secondarily by the disappearance in these places not only of the inter-follicular tissue, but also of the granulosæ and fibrosæ. If *c''* then is not a bud, how did it come to occupy its present position?

In *d*, follicles *d''* and *d'''* are attached to the main suspensorium by short necks of their own. These however are so completely fused proximally with the suspensorium of *d* that their independence is largely obliterated. *E''*, although firmly fused to *e*, really has a suspensorium of its own, but this is reduced to an extremely fine thread, visible as an independent attachment only in one or two places. *C''* upon careful examination proves also to have indications of an independent attachment to the central ovarian mass. The threads therefore are reduced suspensoria, and the compound follicles are fusion products.

The question how these fusions occur remains to be answered. Indications as to how these could have come about are contained in the facts just discussed, for these suggest that one follicle is carried away from the central ovarian mass by another. If this is true, we should be able to discover various stages in the process. Accordingly we can interpret *b* as a late stage in which intimate union has occurred among the follicles distally, whereas proximally the suspensorium of the group shows the multiple nature of its origin by its distinct division into a number of strands. *A* is another late stage, only here the follicles seen are larger than in *b*, their fusion is less complete, and *a''* has worked its way into *a* so that the yolk of the latter came to lie between

the granulosa of the outer follicle and the connective tissue covering of the inner one. In this case, too, dissection clearly exposes the compound nature of the suspensorium. *C*, *d*, and *e* represent stages of fusion less complete than the preceding.

If the suggestions made by these follicles and suspensoria are really correct it ought to be possible to find small follicles not only in places where they are not obvious, but also at various levels of the suspensoria, and this has actually been done. For instance, *a*, prior to dissection, was not obviously double. *J* is

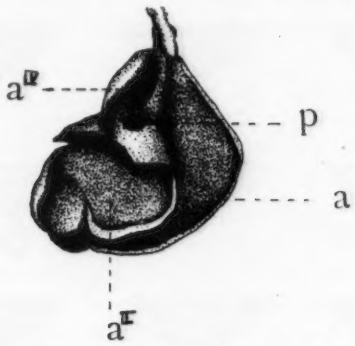


FIG. 2. Dissection of compound follicle, *a*, *a''*, and *a'''*, Fig. 1. Natural size. In this figure *a'''* is omitted. The suspensorium of a^{II} has been opened and an additional follicle a^{IV} is exposed. The pore, *p*, establishes a communication between the follicular cavity of a^{II} and a^{IV}.

not simple and somewhat distorted, but double, one follicle being considerably larger than the other; *i* is triple, having a central follicle, 2, and two equal-sized smaller ones, 1 and 3. The one on the right, 3, communicates with 2 by means of a small irregular aperture. No opening connects 1 and 2. In both *i* and *j* the suspensoria appear to be united, and there seems no way of explaining how the extra follicles got into their present position, than by assuming that the same process brought them there that carried the main follicle away from the ovary.

Most illuminating in this connection is the presence of accessory follicles on the suspensoria. Sometimes these are large enough to be easily seen, as in the case of *a'''*, of *f''*, attached to the neck of *f*, a large empty double follicle, and of *g''*; occasionally

they are quite small, like those on the necks of *c* and *j*, whereas at times they may be completely hidden, as in the neck of *a''*, where another follicle was discovered only after the dissection shown in Fig. 2 had been made.

Given weakness on the part of the tunica albuginea, three minor variations of one and the same process are capable of accounting for these different types of follicles. I have tried to represent this in a series of diagrams (Fig. 3).

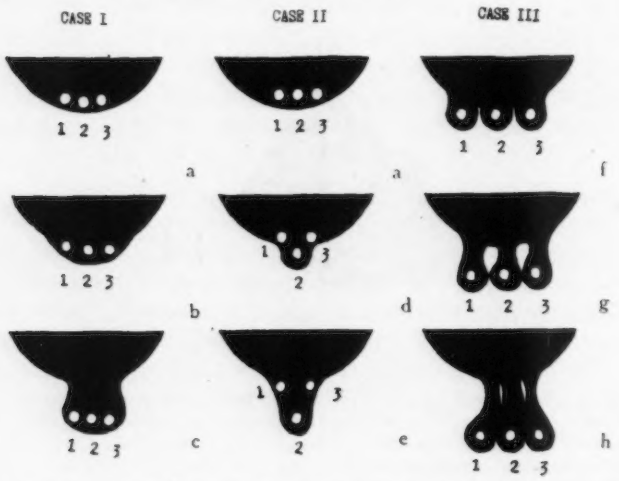


FIG. 3. Diagrams to illustrate the origin of compound follicles. Description in text, p. 182–183.

If we imagine that the ovarian surface evaginates rather broadly beneath egg 2, Case I., diagram *a*, then eggs 1 and 3, which are supposed to lie very close to 2, will be carried away from the ovary, as in *b* and *c*. This would lead to the formation of a follicle of the type *i* and *j*, Fig. 1.

If on the other hand only a very small evagination should take place under egg 2, Case II., its neighbors, 1 and 3, if carried away from the ovary, would not come to lie in such close proximity to 1 in the evagination. In fact they would lie higher up in the suspensorium, *d*, *e*, Fig. 3, and in this manner sufficiently great differences in early distribution would account for the later

development of follicles at various points on the suspensoria as in *a*, *c*, *f*, *j*, etc., Fig. 1.

If, owing to greater distances between the eggs, or to greater stiffness on the part of the tunica, separate evaginations should occur beneath three follicles as in Case III., diagram *f*, Fig. 3, these if not too remote from one another might give rise to a triple follicle, having a common suspensorium except near the ovary where its multiple origin would still be apparent upon close examination. These processes, represented diagrammatically in *g* and *h*, would give rise to complexes similar to *a* and *b*, Fig. 1. If the number of follicles involved were greater, more complicated results would follow. The possibilities of greater complication are clearly indicated by a group of relatively young follicles such as *z*, Fig. 1, as well as by instances in which follicles, of patently distinct origin proximally, have fused distally with others with whom they were brought into contact secondarily by mere accident. Good examples of adhesions of this kind are follicles *x* and *y*, Fig. 1.

In its relation to the production of double-yolked eggs, this ovary is full of suggestions. Follicle *f*, Fig. 1, is an empty double one, and there can be little doubt that it was instrumental in the production of one of the abnormal eggs. Follicles *a* and *a''* contained yolks of essentially the same size, and there seems to be no good reason for doubting that these two would have been shed at the same time. In *b*, the follicles fall into two groups of two each. In one of these they are distinctly larger than in the other, but the follicles within each group are of the same size. It seems likely that in this complex we have the physical basis for the production of two double-yolked eggs in rapid succession. *Z* suggests the possibility of a series of abnormal eggs such as this bird is known to have laid on several occasions.

Physiology of the Ovary.

The morphological findings and their interpretation leave open the question as to how one may picture the physiology of this organ. Follicle *b*, Fig. 1, demonstrates the possibility of relatively independent growth on the part of two groups of eggs, as well as the possibility of synchronism in eggs belonging to the

same group. How this can be is answerable by assuming that the vascular supply to the two groups was different, whereas to the individual members of each group it was practically the same. The physical basis for this might easily be found in the character of the fusion undergone by the individual suspensoria, for if this should be such as to partially occlude the flow of blood in certain directions, the follicles supplied by the vessels involved would lag behind those furnished with a better supply. That the blood supply to certain follicles must be affected, is clearly shown by the suspensoria which are reduced to mere threads.

In certain instances, however, a follicle despite its reduced suspensorium is practically as large as the neighbor to which it is attached, as in the case of *e''*. This suggests the establishment of a secondary blood supply derived from the better equipped follicle. The highly vascular condition of the connective tissue between the granulosæ certainly is favorable to this interpretation. In fact in view of the structural relations it is difficult to see how the blood supply could be otherwise than identical in this case.

Identity of vascularization makes another fact understandable. A follicle such as *c''* would under normal conditions have a blood supply much less than that of its "host" *c*. As it probably is dependent on that of *c*, however, owing to its intimate fusion with it, and the reduction to almost nothing of its own suspensorium, it is likely to have more material brought to it than it can metabolize in normal fashion. This follicle, as well as others of its size similarly placed, showed considerable depositions of yolk in the lacunæ of their hypertrophied granulosæ.

This fact is not only suggestive as to the rôle played by the granulosa during oögenesis, but also of the part of the egg itself in initiating the deposition of yolk, for if the ovum in this instance is not supposed to have been less permeable to the raw materials of yolk than the one in the larger follicle, where only traces of yolk could be found in the granulosa, it is difficult to see why the two eggs should have been unequal in size. There must be a period in the ovarian life of an egg when its permeability to certain substances is suddenly greatly increased. Riddle's work ('11) suggests the same thought, but this abnormal ovary

indicates that the conditions followed by increased permeability are to be looked for in the egg. If ova in the same state have an identical blood supply they will undergo equivalent growth, but mere identity in their circulation does not insure this.

Although these considerations are necessarily speculative, their possible relation to more remote matters may be briefly mentioned. It is certainly a remarkable fact that in the human ovary, to mention a case among mammals, and in the hen's ovary, to mention one among birds, normally one egg ripens after another, whereas in the ovaries of amphibians and fishes thousands of eggs may ripen at one time. It is quite possible that the conditions which in the one case lead to an abnormality are identical with those which in the other give a typical result. In no case do all the eggs contained in any of these ovaries ripen at the same time. This certainly points to alterations in the permeability of the ovum as a factor in the initiation of its growth period, and suggests that these changes are directly traceable to the activity of the egg. The fusion of normally distinct follicles in the hen's ovary brings about secondarily an identical vascularization and thus accidentally duplicates the nutritive conditions prevalent in the ovaries of certain other forms.

Identity of blood supply then is suggested as an explanation of the synchronic yolk formation in these eggs, whereas a change in permeability undergone independently on the part of the eggs themselves introduces this period of growth. This change in permeability must necessarily also be synchronic if double-yolked eggs are to be produced, but how this synchronism is brought about in certain eggs, and how it is prevented in others, cannot be profitably discussed in our present state of knowledge.

The important thing after all is that a bird's ovary, with follicles secondarily fused as described in the preceding pages, does actually give rise to a series of double-yolked eggs, and this remains true even if my suggestions as to the development and physiology of this organ should prove to be entirely wrong. It is to be hoped that the clinical symptoms given may aid in the discovery of further material upon which more detailed studies in the living state could be carried out with great advantage to

our knowledge of the more obscure points in the physiology of egg-production.

ZOÖLOGICAL LABORATORY,
UNIVERSITY OF MICHIGAN,
November 22, 1912.

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PRELIMINARY NOTE ON THE RESULTS OF CROSSING TWO HEMIPTEROUS SPECIES WITH REFERENCE TO THE INHERITANCE OF AN EXCLUSIVELY MALE CHARACTER AND ITS BEARING ON MODERN CHROMOSOME THEORIES.

KATHARINE FOOT AND E. C. STROBELL.

Interest in the studies of the chromosomes has been greatly stimulated during the past few years by the revival of the hypothesis that they are the bearers and distributors of all hereditary characters. Belief in such a fundamental significance of the chromosomes has led its firmest adherents to interpret every phase of their morphological and physiological expressions into terms of a causal nature, even to the point of claiming that definite chromosomes—such as the so-called sex-chromosomes—are the determining factors of sex and of all sex-linked characters.

On the other hand a large number of cytologists have studied the chromosomes from an entirely different point of view—believing that their morphological phases are not to be interpreted in terms of a causal nature, but like many other organs of the cell, they are the expression rather than the cause of cell activities.

These opposing interpretations can be strikingly demonstrated by a few quotations from recent papers. Morgan ('11) writes: "The experiments on *Drosophila* have led me to two principal conclusions: First, that sex-limited inheritance is explicable on the assumption that one of the material factors of a sex-limited character is carried by the same chromosomes that carry the material factor for femaleness.

"Second, that the 'association' of certain characters in inheritance is due to the proximity in the chromosomes of the chemical substances (factors) that are essential for the production of those characters" (pp. 365).

Wilson ('12) gives the stamp of his approval to Morgan's

conclusions. He says Morgan's results "bring strong support to the view that the chromosomes are such bearers of unit-factors, for the whole series of phenomena determined in *Drosophila*, complicated as they seem, become at once intelligible under the assumption that certain factors necessary for the production of the sex-limited characters are borne by the *X* chromosome; and without this assumption they are wholly mysterious" (pp. 420–1).

As opposed to these definite expressions of faith in the causal nature of the chromosomes, we may quote Child's latest repudiation of all such hypotheses. "Let us take the case of the chromosome, for example, which plays so important a part in recent biological hypothesis. What is the chromosome? If it is what many authors seem to believe, it is an autonomous being endowed with something more than human intelligence. But if we are not willing to believe this, then we must regard the chromosome as an incident or result of dynamic processes in the organism, like other morphological entities. If this is the correct view, then it is nothing ultimate or fundamental. We must analyze it into terms of the processes which have made it, and in this analysis we shall sooner or later find nothing more or less than the whole complex of processes which constitute the organism. The organism makes the chromosomes, not the chromosomes the organism" (pp. 33).

Our cytological studies have caused us to sympathize with the many investigators who have expressed skepticism of the causal nature of the chromosomes. For several years we have argued that the chromosomes in the forms we have studied show too much variability, both in their morphological and physiological expressions, to justify those theories which obviously demand a rigid compliance to a definite mode of expression. We demonstrated in 1905 that the form and relative size of the chromosomes in *Allolobophora fœtida* are inconstant and in every publication since that date we have demonstrated variability in the form, relative size and behavior of the chromosomes in every form we have studied, and we have consistently argued that such variability attacks the very foundations upon which the popular chromosome speculations of this decade have been built.

Recent experimental results have caused a marked modification of the views of some of the adherents of the more extreme chromosome hypotheses, forcing them to modify the theory of the individuality and continuity of the chromosomes so far as to admit that there must be an interchange of chromatin between individual chromosomes and that the chromosomes which emerge from synapsis are "probably not identical with the original conjugants" (Wilson, '12, pp. 422). They do not however extend this interpretation to the XY chromosomes: "the degree of union may vary in different cases, involving sometimes no fusion, as is suggested by the history of the XY pair" (Wilson, '12, pp. 417). This would seem to be an inevitable conclusion, otherwise any facts that could be assumed to be explained on the supposition of an interchange of factors between X and Y, would be inexplicable for those forms in which no Y is present.

In analyzing the results of our recent experiments we shall accept, for the sake of the argument, the above assumption that there is no interchange of material between the XY chromosomes, and also the hypothesis of male- and female-producing spermatozoa.

In 1909 Castle suggested that the Y chromosome of those forms in which this morphological element is present may be the bearer of all characters that are exclusively male. He wrote: "I would offer the suggestion that we have a mechanism suitable for the transmission of characters exclusively male in the Y element described by Wilson, the 'synaptic mate' of the X element."

It would seem possible to test the value of this suggestion by crossing two species, each having the XY chromosomes, and one of the species having an exclusively male character which is lacking in the other. These conditions are met in the two Hemipterous species *Euschistus variolarius* and *Euschistus servus*. The former has a character that is exclusively male, in the form of a distinct black spot on the male genital segment, while such a spot is entirely lacking on the male genital segment of *Euschistus servus* (Photo 1). There is no black spot on the female genital segment of either species.

During 1911 and 1912 we succeeded in crossing three species

of Hemiptera, but in this preliminary note we shall confine ourselves to discussing the cross between *Euschistus variolarius* female and *Euschistus servus* male. From this cross we were able to raise to maturity 11 males and 16 females of the F_1 generation and these fortunately mated readily and proved to be very fertile. Seven of these pairs were isolated and the offspring of each pair raised in separate cages—in many cases even the bugs from a single batch of eggs being isolated during the entire period of their development. From these seven pairs of the F_1 hybrids we raised 249 females and 204 males. These bugs were kept in the laboratory until several days after they had reached the winged stage, when the females were preserved as pinned specimens and 191 of the males were preserved in glycerine, as shown in photos 2[1] to 6.

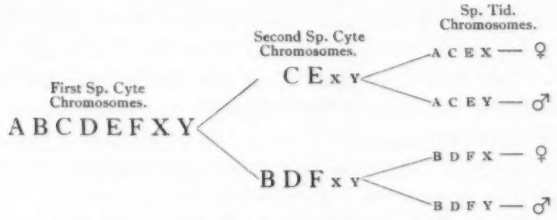
An analysis of the maturation divisions would seem to support Castle's suggestion that the *Y* chromosome may be the bearer of exclusively male characters, for this chromosome is the only one that can be in both the so-called male-producing spermatozoa of each quartette of spermatids resulting from the two maturation divisions, assuming that these divisions occur as illustrated in the text-figure.

It would seem quite logical to conclude that if a character associated exclusively with the male sex can be inherited from the father, the factors which produce it should be in the so-called male-producing spermatozoön and if they are to be located in a given chromosome it must be the one and only chromosome that *ex hypothese* can be present in all the so-called male producing spermatozoa, otherwise the character would not be a constant feature.

If Castle's suggestion is correct, then all characters exclusively male can be inherited only through the father, as the *Y* chromosome is never in the female. If then *Euschistus variolarius* is fertilized by *Euschistus servus*—a form which lacks the spot—none of the offspring should have it, neither the F_1 nor the F_2

[1] We have photographs of all the male hybrids, but they must be reproduced by the bromide print method in order to secure an accurate reproduction of the shades of difference in the size and intensity of the spot. We hope to be able to reproduce all these photographs when we publish the detailed account of the crossing of the three species, *Euschistus variolarius*, *Euschistus servus* and *Euschistus ictericus*.

generation. *As a matter of fact, however, the spot of Euschistus variolarius is transmitted through the female—appearing in a slight degree in the males of the F_1 generation and much more intensely in the F_2 generation*—some of the offspring of this generation having the genital spot quite as conspicuous as that of their maternal male ancestors—*E. variolarius*. Such facts would seem to dispose beyond question of Castle's suggestion that the factors which produce exclusively male characters must be located in the *Y* chromosome.



Scheme of the two maturation divisions of *Euschistus variolarius* and *Euschistus servus* based on the assumption that the first maturation division separates autosomes of maternal and paternal origin and the second division halves them. The *XY* chromosomes on the contrary being halved in the first division and separated in the second division. The relative positions of the autosomes may be changed unless definite chromosomes are always destined to the same pole, but reversing their position in this regard does not alter the end result—that the only chromosome common to both so-called male-producing spermatids is the *Y* chromosome.[1]

We may next examine the facts on the basis of the assumption that the factors which produce the male genital spot can be located in the *X* chromosomes. If this is true then the spot cannot be transmitted through the so-called male-producing spermatozoön for this spermatozoön contains no *X* chromosome.

We hoped to be able to test this by fertilizing a pure female *E. servus* by a pure *E. variolarius*, but owing to great scarcity of material in the locality where we were working we were unable to find any male *variolarius* at the time it was possible to attempt this experiment. We were, however, able to cross a pure *E. variolarius* male with an F_1 female and compare the male offspring with those obtained by the F_1 matings.

[1] We have used the method of designating univalents by the letters of the alphabet, bivalents being represented by A B, C D, E F. Only six of the twelve autosomes are designated.

If, as stated above, we attempt to locate the spot factors in the *X* chromosomes, we cannot expect these factors to be transmitted through the male producing spermatozoon and therefore if an F_1 female hybrid is fertilized by a pure *E. variolarius*, we should expect to have a no more pronounced spot in the offspring than we obtain by matings of two F_1 hybrids. *The facts, however, demonstrate that the spot is transmitted through the pure male E. variolarius far more intensely than it is through the F_1 male.*

This is strikingly illustrated by a comparison of the male offspring of an F_1 female and pure male *variolarius* with the 191 males we have succeeded in raising from seven pairs of the F_1 hybrids. We have 18 males from the former pair and many of them have the genital spot stronger than any of the 191 males from the F_1 parents. Further, none of these 18 males are without any spot, *whereas 84 of the 191 males of the F_2 generation have no spot whatever.*

Our facts seem to have demonstrated two points: First, that the spot is inherited through the female without the aid of the *Y* chromosome, and second, that the spot is inherited through the male without the aid of an *X* chromosome. We seem forced then to admit that in this case neither of the so-called sex chromosomes is necessary to the inheritance of an exclusively male character, the factors of which, it would seem, ought to be contained in a chromosome which determines sex, if, in fact, sex determining chromosomes exist. The spot in *E. variolarius* is a part of the male genital segment and it is only logical to assume that the factors which produce it are associated with the factors which produce the male genital segment itself, wherever or whatever those factors are. As the facts do not seem to warrant the assumption that these factors are confined to either of the so-called sex-chromosomes, we may re-examine them in the light of placing these factors in some other chromosome or chromosomes.

As the so-called male-producing spermatozoön of many forms has no *Y* chromosome and therefore no male sex-chromosome, it is obvious that if this spermatozoön is assumed to carry a factor which produces sex, this factor must be placed in some other chromosome, by those who believe that the chromosomes

are the vehicles of such factors. Realizing this Morgan ('11) concluded that "the factors for producing the male must be located in some other chromosome" and he places these factors in a pair of homologous chromosomes, as shown by his scheme.

Gametes of female — X M—X M
Gametes of male -- X M—M.

A glance at text-fig. 1 will show that placing the male factors in an homologous pair of chromosomes (in *A* and *B* of Fig. 1 for example) necessitates their presence in all the spermatozoa of both sexes, thus giving to the so-called female-producing spermatozoön the male-producing factors in addition to the female-producing factors. The female-producing spermatozoön is therefore heterozygous for sex and the male-producing spermatozoön homozygous for sex—and this seems to involve a denial of the assumption that has been the basis of so much experimental analysis, namely, that the female is homozygous for sex. How can the female be homozygous for sex when the so-called female-producing spermatozoön has conveyed to her "the factors for producing the male"?

If we assume with Morgan that both members of a definite diploid pair of chromosomes contain the factors for producing a male, this must hold for the same diploid pair in both the female and the male, and if the factors producing exclusively male characters are linked with the factors for maleness, then each member of this diploid pair should contain the factors for producing the spot, which in *E. variolarius* is an exclusively male character.

As this diploid pair is in the female as well as in the male, it would seem necessary to assume that in *E. variolarius* the female carries an inhibitor of such exclusively male characters. But one is embarrassed in considering where to place this inhibitor—if we locate it in both members of an homologous pair of chromosomes, then it would be in the male as well as in the female. If we place it in only one member of an homologous pair (in *A*, for example, of text-fig. 1), this would involve selective fertilization, for if the female is fertilized by the so-called female-producing spermatozoön that is without the *A*, and she has by

chance discarded the other *A* in her polar body, she also would have the spot, and further, half the male producing spermatozoa have the *A* chromosome and therefore they must not function or the spot would be absent in half the variolarius males.

If we place the inhibitor in the three *X* chromosomes, the assumption obviously would not work, for the male has one of the three *X* chromosomes and the spot would therefore be inhibited in the male as well as in the female. It would therefore be necessary to assume that in *E. variolarius* two doses of inhibitor are necessary to cancel the spot. This assumption, which apparently would hold for *E. variolarius*, is, however, found to be untenable if used to explain the results of the cross between *E. variolarius* and *E. servus*, for the female hybrid cannot receive a double dose unless we assume that *E. servus* carries an inhibitor for inhibiting in the female a male character which is never present in the male. If, however, we feel justified in assuming that the female hybrid has a double dose of inhibitor, this would fail to explain the absence of the spot in those male hybrids, which do not have it, for they have only one *X* chromosome and therefore only one dose of inhibitor. We might avoid this by assuming that one dose only would be necessary for the female hybrid, as she presumably has only one dose of spot factors; but this would involve inhibiting the spot in all the F_1 males also, which is opposed to the facts, as the spot is not inhibited in all the F_1 hybrids. The facts show that the spot factors—whatever they are—are latent in the female and if they are suppressed by an inhibiting factor, it would seem that this cannot logically be located in a definite chromosome.

Our results demonstrate that the spot is by no means a unit character. If we speak of it in terms of Mendelism we must say that it is the result of a large number of unit factors, for in the 107 males of the F_2 generation in which a spot can be identified, it is present in every degree of intensity, from a mere indication of a spot to those which are nearly, if not quite, as conspicuous as the spot of a pure *variolarius* (Photos 2–4).

If we assume that each member of one diploid pair of chromosomes contains all or half the factors for the genital spot, in addition to the factors for maleness, then the ripe egg of *E.*

variolarius contains one such chromosome and it follows when the egg is fertilized by *E. servus* it will have one diploid pair of chromosomes that is homologous for the factors producing a male, but heterozygous for the factors producing the spot.

Such a diploid pair should be present in all the offspring—both males and females, and if we cancel it in the females by assuming an inhibitor somewhere, we still have it in the males and therefore the spot should be quite as pronounced in the F_1 hybrid males as in *E. variolarius*. The facts, however, are as follows: None of the 11 male hybrids have a spot as strong as *E. variolarius*, 2 have no spot whatever, 3 have a faint spot—like that of the third bug from the bottom of the tube in photo 4—4 have a slightly stronger spot, and 2 have a spot about one-third as pronounced as that of *E. variolarius*. The genital spot in the F_1 hybrids is very variable, it is not expressed as a dominant, a recessive or a true blend.

If we attempt to explain these results in terms of chromosomal distribution of the factors, we encounter serious difficulties and are forced to assume the illogical position of suggesting a method of chromosome division for the hybrids which obviously does not occur in *E. variolarius*, as the spot is not variable in this form.

Assuming that the diploid pair of chromosomes in the egg of *E. variolarius*, which was fertilized by *E. servus* is heterozygous for the spot factors (that they are in chromosome *A*, for example, of the pair *AB*), then it must be asked why those in chromosome *A* are completely suppressed in some of the F_1 hybrids, while part of them find expression in other hybrids.

We might simply assume that some of the factors of chromosome *A* have dropped out, but the facts show this to be untenable for the spot reappears in the F_2 generation—in some cases quite as pronounced as in the pure *E. variolarius*. We must thus assume that the female *variolarius* has at least half the spot factors which she transmitted to the male hybrids, although these F_1 hybrids show either no spot at all or an incomplete spot. To account for these facts we must assume that the male hybrids differ from the pure males in having an inhibitor that inhibits those spot factors which are present but which are not expressed. We have seen that the facts will not allow placing the

inhibiting factors in either the X chromosomes or the ordinary chromosomes and we are thus forced to admit that inhibiting factors—whatever they are—must be located outside the chromosomes—in the region of pure hypothesis. The facts force us to consign to these hypothetical inhibitors, not only the responsibility of suppressing the spot factors in all the females, but also of determining just how many spot factors shall find expression in the males of the F_1 and F_2 generations, and thus they practically relieve the chromosomes of the burden of unit distribution.

Chromosomes.—The small idiochromosome is an exclusively male character—a feature it has in common with the genital spot—and if it could be put to the same test of experimental breeding, which we have attempted for the genital spot—the results ought to show whether this idiochromosome has indeed the individuality which modern chromosome theories demand or whether—like the genital spot—its relative size is merely a structural feature which can be transmitted through the female. We confidently expected to be able to apply this test to the small idiochromosome of *variolarius*, for it had been demonstrated that this chromosome in *variolarius* was relatively much smaller than in *servus*.

The chromosomes of *Euschistus servus* were studied by Wilson in 1906 and he concluded that the XY chromosomes of this form differed from those of *E. variolarius* in their relative size. He says: "The above described species of *Euschistus*, while agreeing precisely in the general relations, present individual differences so marked as to show that even the species of a single genus may be distinguishable by the chromosome groups. In this case the most interesting feature is the series shown in the inequality of the idiochromosomes, which become progressively greater in the series (1) *E. servus*, (2) *tristigmus*, *fissilis*, (3) *ictericus*, (4) *variolarius*, the inequality in the last case being fully as great as *Lygæus*" (pp. 17). He demonstrates a marked difference in the relative size of the idiochromosomes of *E. variolarius* and *servus* in *a* and *c* of his Fig. 4.[1]

[1]We have a large number of photographs of entire groups of chromosomes of *E. variolarius* and *E. servus* and we have also preparations from which we can photograph the chromosome groups of the F_1 and F_2 generations of this cross from both the testes and embryonic stages, but these results are reserved for a later publication, in which we shall give a complete account of our experiments in crossing *E. variolarius* with both *E. servus* and *E. ictericus*.

Hoping that such a marked difference in the chromosomes might be a constant feature, we anticipated being able to obtain some interesting comparative results by crossing the two species. We were disappointed, however, to find that in all the individuals we studied the inequality in the size of the idiochromosomes of *E. servus* is quite as marked as in *E. variolarius*, and that the chromosomes of these two species were therefore of no special value for this comparative study.

This seems to be another case pointing to inconstancy in the morphology of the chromosomes—as the individuals which Wilson studied showed a marked feature which was absent in the individuals studied by us. All such examples of inconstancy in vital processes throw additional light on the fact that investigators of the same material so frequently hold diametrically opposed views as to the fundamental significance of such expressions of vital activities, and that facts in support of these opposing views can be demonstrated in the same material.

This is almost amusingly illustrated by two recent papers on the cytology of certain Hemiptera, Gross ('12) and McClung and Pinney ('12). Gross differs from Wilson as to the facts on almost every point in which their hypothetical views clash. Of the cytological evidence for Wilson's theories, he says, "Geleitet von einigen Gesichtspunkten, die ich bei der Untersuchung von *Syromastes* und *Pyrrhocoris* gewonnen hatte, habe ich aus dem Studium der Literatur den Eindruck erhalten, das die scheinbar so sichere cytologische Basis von Wilson's Theorie hochst unzuverlässig ist. Ja, man kann mit gutem Grunde sagen, sie existiert eigentlich gar nicht."

He denounces Wilson's work on *Syromastes* as follows:

"Im einzelnen enthält Wilson's recht kursorische Darstellung zahlreiche, leicht nachzuweisende Lücken, Ungenauigkeiten und Irrtümer."

McClung ('12) condemns our work on *Anasa tristis* with quite as firm a hand, attributing to our methods of technique our failure to demonstrate the facts which he considers of most value. With the finality of a judge he hands down his decision: "It is my judgment that this method '*used alone*' is entirely inadequate for accurate results, and in this particular case is

responsible for the discrepancy between these investigators and Paulmier, Wilson and Montgomery."[1]

A few facts will serve to convince any unprejudiced cytologist that our method of technique should not be condemned as "inadequate." From many of our preparations it is possible to demonstrate a large number of chromosome groups in which every chromosome is present and can be clearly photographed—in one case we have more than 150 photographs of such groups from the *same embryo*. A method that makes such results possible can hardly be condemned as "inadequate" for "accurate results"—it is a method, rather, that compels a recognition of enough variability in the same individual to make one very cautious in accepting premature hypotheses based on insufficient data.

In further condemnation of our methods McClung criticizes the value of photography as a means of demonstration. He says: "Foot and Strobell have employed photography alone as a means of presenting illustrations of their material, and it is assumed by them that if a thing can be photographed it must necessarily be a true picture of normal conditions. This I consider to be a decided fallacy. A photograph is an interpretation by the observer, just as is a drawing. The personal factor is no more absent from one method of illustration than it is from the other. Photographs may present with greater fidelity the details of structure in an object, but the choice of the object, and the nature of details are at the command of the photographer" (pp. 369).

We certainly do *not* assume that a photograph must of necessity represent only normal conditions, but we do believe that abnormalities are likely to be due to a pathological condition of the cells and that such a condition can quite unconsciously be obscured in a drawing, but not in a photograph. McClung's position is certainly unique when he claims that the personal factor is no more absent from a photograph than from a drawing and he bases this on the fact that an investigator may in either case *select* the object he wishes to photograph.

[1] It is difficult to understand why McClung mentions and underscores "*used alone*" as on the same page he quotes from our paper in which we distinctly state that we use sections also for "comparative work."

It may be said that in every paper representing cytological research one or more hypotheses are on trial and it is certainly the privilege of the investigator to select for illustration such evidence in his preparations as support his own convictions in regard to the hypotheses under consideration.

Our skepticism of the sex-determination theory led us to study our preparations of *Anasa tristis* with a view to determining whether the phenomena involved in the theory were sufficiently constant to justify it. We found enough evidence to fully confirm our skepticism and we claim that we had the right to select that evidence from our preparations and give it, with as much emphasis as possible.

The facts are simple and clear. We demonstrated that in *Anasa tristis* the reverse of the facts demanded by the sex-determination theory were clearly present and could be illustrated by photographs. The interesting feature in these opposing observations is the fact that we do not deny the observations of our opponents, whereas their belief in the causal nature of the chromosomes compels them to set our observations aside or to resort to strained explanations in order to account for them.

It is the old story so familiar to cytologists—if a feature is where, hypothetically, it ought not to be, it is an artifact, and if it is not where it ought to be, it is due to faulty technique.

We believe that the photographs of our preparations prove conclusively that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle and the case may safely rest on a comparison of McClung's photographs of his preparation with ours.

We claim that the facts we have demonstrated in *Anasa tristis* are at least worthy to be placed in evidence against the chromosome theories that necessitate such facts being thrown aside as artifacts or as expressions of a pathological condition. We hold that they have at least the same value as normal variations in other organs of the cell and that they should be accepted as the same type of evidence we gave in '05 against the theories based on definite forms of the chromosomes. We demonstrated a degree of inconstancy in the form of the chromosomes of *Allolobophora fœtida* that should not exist if the hypotheses were valid.

It is interesting that such an experienced student of the cytology of the Hemiptera as Gross ('12) should also find that there are facts in *Anasa tristis* that are out of harmony with the theories. He writes:

"Später bin ich dann durch die Freundlichkeit der Herrn Dr. E. R. Downing noch in den Besitz einiger Präparate von *Anasa tristis* gelangt, deren Studium mir gleichfalls zeigte, das Wilson's Theorie mit den Tatsachen schwer vereinbar ist."

Nucleolus.—In the few Hemiptera we have studied we have interpreted the chromatin nucleolus of the first spermatocyte as the homologue of the nucleolus of other forms.

This is due to the fact that we have failed to find any other structure in the cell which we have felt justified in interpreting as a nucleolus. We were wrong in interpreting the chromatin nucleolus in *Anasa tristis* as independent of the accessory chromosome, an interpretation we were first led to doubt in our study of *E. variolarius* '09, and we stated in giving our results in this form that we would reserve the publication of the evidence until we could control it by a comparison with other forms. Buchner's work ('09) on the accessory chromosome enabled us to harmonize our conflicting evidence on this point. We wrote: "In this paper Buchner's observations on the accessory chromosome appear to throw some light on certain conflicting facts observed by us in *Euschistus variolarius*. Buchner supports Wassilieff in observing that only part of the substance of the chromatin nucleolus gives rise to the accessory chromosome. This would seem to indicate that the chromosomes in question are evolved from a nucleolar mass of chromatin, thus homologizing this structure with the cases in which it is claimed all the chromosomes are evolved from a large nucleolus, leaving a nucleolar residue after the chromosomes are formed. In *Euschistus* we find cases in which both the idiochromosomes and a chromatin nucleolus are present at the same time. Such facts, added to those cases in which the size relations of the chromatin nucleolus do not agree with those of the idiochromosomes, raise the question as to the identity of the two structures, though these facts would not conflict with homologizing the chromatin nucleolus with the nucleolus, which in some forms is said to give rise to all the chromosomes."

If these investigators are right in claiming that the chromatin nucleolus is in fact a chromosome which retains its individuality and continuity through the growth period, then it is quite necessary that another structure should be found in these cells which can be homologized with the nucleolus of other forms. If one is obviously not present, some plausible explanation of its absence must be given. If it is not figured in drawings, then the observer can be accused of superficial and careless observation; but if it is absent in photographs, then the old scapegoat—faulty technique—is held responsible.

This is McClung's explanation of the fact that a second nucleolus is conspicuously absent from all our photographs of the growth period of the spermatocytes of *Anasa tristis*. He says: "Owing to the technique employed by them the plasmosome is practically destroyed." It is certainly highly improbable that a method that has not destroyed the plasmosome in the other forms we have studied should destroy this structure in these Hemiptera.

It is interesting to notice how the plasmosome of *Anasa tristis* has developed and waxed strong since the earlier investigators described and figured it. It was described as a pale body and figured accordingly, but in Pinney's recent drawings it has developed into a strong dense body fully as chromatic as the chromatin nucleolus. Although Wilson himself describes it as a "*pale*" body, McClung's photographs from Wilson's preparations show that the structure which McClung interprets as a plasmosome is not a pale body, but is fully as chromatic as the chromatin nucleolus. Here, as in other points where our conclusions are condemned, we are willing to rest the case on a comparison of McClung's photographs with ours.

Our belief that the chromatin nucleolus of the Hemiptera is the homologue of those nucleoli of other forms that are said to give rise to all the chromosomes has been greatly strengthened by our study of the spermatogenesis of *Euschistus crassus*. In this form there are two chromatin nucleoli, one of which gives rise to the idiochromosomes and the second gives rise to a pair of autosomes—the so-called ordinary chromosomes.[1]

[1] These results were sent to press last February.

All such deviations from a method of development that has been claimed to be sufficiently rigid to justify far-reaching generalizations are a warning against formulating general laws that do not take into account all the facts.

If our results demonstrated in *Anasa tristis* be shorn of all their significance except their admission as normal variations, they still stand as a protest against premature generalizing. We expressed this in an earlier paper (1911) in reference to our demonstration that the accessory chromosome in *Anasa tristis* does not always fail to divide in the second spindle. "If the significance holds which has been attributed to the failure of this chromosome to divide in one of the two maturation divisions, then those cases in which it divides twice in *Anasa* must be set aside as pathological, the spermatozoa resulting from these divisions having no functional activity. Only to those who have endowed the chromosomes with causal attributes can variations in their behavior cause embarrassment. If we find them subject to marked variations it is a characteristic they have in common with other structures in the cell—the nucleolus, the centrosome, the mitochondria, the polar rings and other structures which have been found to be so variable that interest in speculations as to their possible causal significance has steadily waned, and those who believe the chromosomes are equally variable may justly suspect that the hypotheses surrounding these structures may be destined to the same fate as the speculations so long surrounding other cell organs, notably the centrosome."

Nearly all cytologists agree that experimental breeding has been and may ever be the most trustworthy test of hypotheses which are based on the morphology of the cell and which claim to offer a mechanical explanation of heredity.

The chromosomes of the Hemiptera are largely responsible for the modern chromosome hypotheses, for certain stages in their growth and development which are assumed to justify these hypotheses can be clearly followed and demonstrated. Those Hemiptera therefore which show the chromosome phenomena necessary to a given hypothesis are especially fitted to test its value by experimental breeding.

It would seem that a cross between two species of these Hemiptera, one of which contains an exclusively male character which is absent in the other, should furnish some trustworthy evidence for or against the hypothesis that the factors for determining sex are located in a definite chromosome of the so-called male and female-producing spermatozoa.

We believe that our results from this experimental work are in harmony with our cytological evidence on this point and that these results support the skepticism of those investigators who believe that the evidence given by the chromosomes does not warrant the assumptions that have endowed these cell structures with causal attributes.

NEW YORK,
December, 1912.

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Description of Plates.

In all cases only the ventral surface of the bugs is shown. The specimens in photos 2 to 6 are preserved in glycerine. The genital segment of each bug has been pulled out and cotton inserted behind the segment to hold it in position to show the entire ventral surface. The bugs are magnified about 1½ diameters.

The photographs are reproduced by the half-tone method which, unfortunately, is not entirely satisfactory for showing the delicate shades of difference in the intensity of the spot.

Photo 1. *Euschistus variolarius* male, showing the ventral surface and the clearly defined black spot always present on the genital segment of the males of this species.

Euschistus servus male, showing ventral surface and the genital segment without any trace of the black spot characteristic of *E. variolarius* males.

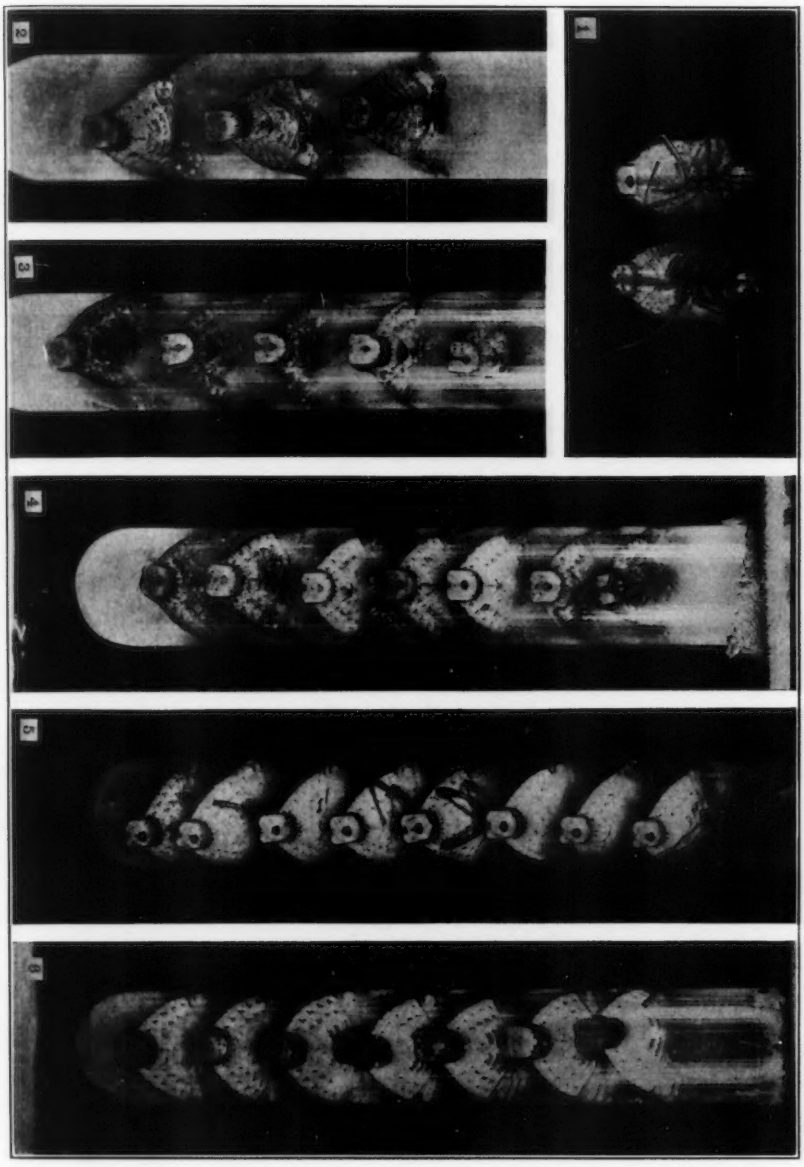
Photos 2 and 3. 8 of 46 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female raised in our laboratory, from a pair collected in Connecticut, and an *E. servus* male collected in North Carolina. Four of these F_2 individuals have no black spot on the genital segment, and four show the spot in a greater or less degree.

Photo 4. 7 of 54 males of the F_2 generation raised from a pair of F_1 hybrids, which were bred from an *E. variolarius* female collected in Connecticut and an *E. servus* male collected in North Carolina.

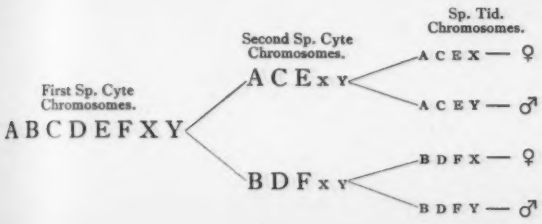
These F_2 individuals show the variations, from no spot on the genital segment, to one almost as pronounced as that of a pure *variolarius*.

Photo 5. *Euschistus variolarius* males, showing the typical black spot on the genital segment.

Photo 6. *Euschistus servus* males, showing the genital segments without the spot characteristic of *E. variolarius*.



FOOT AND STROBELL.

ERRATUM.

Substitute this for the table on p. 191, Vol. XXIV.; paste in place.